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(54) Title: METHOD AND NUCLEIC ACIDS FOR THE ANALYSIS OF A LYMPHOID CELL PROLIFERATIVE DISORDER

(57) Abstract: The present invention relates to modified and genomic sequences, to oligonucleotides and/or PNA-oligomers for detecting the cytosine methylation state of genomic DNA, as well as to a method for ascertaining genetic and/or epigenetic parameters of genes for use in the differentiation, diagnosis, treatment and/or monitoring of lymphoid cell proliferative disorders, or the predisposition to lymphoid cell proliferative disorders.

Field of the Invention

The levels of observation that have been studied by the methodological developments of recent years in molecular biology, are the genes themselves, the translation of these genes into RNA, and the resulting proteins. The question of which gene is switched on at which point in the course of the development of an individual, and how the activation and inhibition of specific genes in specific cells and tissues are controlled is correlatable to the degree and character of the methylation of the genes or of the genome. In this respect, pathogenic conditions may manifest themselves in a changed methylation pattern of individual genes or of the genome.

The present invention relates to nucleic acids, oligonucleotides, PNA-oligomers, and to a method for the analysis of lymphoid cell proliferative disorders, the differentiation between subclasses of said disorder or the detection of a predisposition to said disorders, by analysis of the genetic and/or epigenetic parameters of genomic DNA and, in particular, with the cytosine methylation status thereof.

Prior Art

Lymphomas, e.g. solid tumours of lymphoid cells, fall in two distinct groups: Hodgkin's lymphoma and Non-Hodgkin's lymphoma (NHL). In the United States the incidence of NHL in the year 2000 was estimated to be 55 000 cases, which resulted in an estimated 26,000 deaths.

NHL accounts for 5% of new cancers in men and 4 % of new cancers in women each year in the US and is responsible for 5% of deaths. In 1997 NHL was reported to be the leading cause of death from cancer in men between the ages of 20 and 39 (Greenlee et al., Cancer J Clin 2000, 50:7).

Non-Hodgkin's lymphoma (NHL) can be further subclassified into different classes according their origin from different differentiation states of B or T lymphocytes.

For B-cell NHLs the following classes can be distinguished on a cytological basis:

- chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL, originating from the mantle zone of a lymphoid follicle)
- mantle cell lymphoma (MCL, originating in the mantle zone of a lymphoid follicle)
- follicular lymphoma (FL, originating in the germinal centre of a lymphoid follicle)
- diffuse large B-cell lymphoma (DLBCL, originating in the germinal centre of a lymphoid follicle)
- lymphoplasmacytoid lymphoma (LPL, originating in the germinal centre of a lymphoid follicle)
- Burkitt's lymphoma (BL, originating in the germinal centre of a lymphoid follicle)
- mucosa-associated lymphoid tissue lymphoma (MALT, originating from the marginal zone of a lymphoid follicle.

Correct classification of NHL subtypes is essential as clinical outcome and therapy options vary greatly among the subclasses. In particular the correct identification of mantle cell lymphomas among other small B-cell lymphomas is clinically relevant because mantle cell lymphoma is a far more aggressive disease and displays significantly shorter survival than other histologically related forms (Berger et al., Blood 1994, 83;2829, Fisher et al., Blood 1995, 85:1075). CLL/SLL, which arises from the same zone of the lymphoid follicle as the mantle cell lymphoma and often resembles mantle cell lymphoma regarding morphology, usually progresses rather slowly and is traditionally treated with chlorambucil and cyclophosphamide. In contrast, MCLs show an overall survival of 3 years with a failure-free survival of 1 year after treatment. MCL is typically treated with regimens containing cyclophosphamide, doxorubicin, vincristine and prednisone. Because of the poor outlook young patients with mantle cell lymphoma often undergo autologous or allogeneic bone marrow transplantation (Stewart et al., Ann Oncol 1995, 6: 263). More recently, MCL can be treated with the monoclonal antibody rituximab (Coiffier et al., Haematologica. 1999, 84:14-8.).

Each of these neoplasms has a characteristic morphology, which may be sufficient in a given case to permit diagnosis and classification on morphologic grounds alone, if well-prepared sections are available. However, histologic diagnosis of malignant lymphoma requires much expertise and in cases which present with an atypical morphology classification cannot be achieved on histological grounds alone. In these cases additional markers such as immunophenotyping and cytogenetics are essential to classify the lymphoma correctly, in most other

cases these methods are helpful (Armitage et al., Principles & Practice of Oncology. De Vita VT, Hellmann S, Rosenberg SA. 6th Edition).

In the last 20 years significant progress has been made to characterise these subclasses on a cytological, cytogenetic and molecular level. It could be shown that most of these genetic lesions selectively associate with specific NHL subtypes (Harris et al, Blood 1994, 84;1361, Harris et al., J Clin Oncol 1999, 17: 3835). Knowledge of specific molecular alterations has improved the system of classification and helps to categorise patients in a more clinically relevant way.

Molecular markers offer the advantage that even biopsy samples of very small sizes and samples whose tissue architecture has not been maintained can be analysed quite efficiently. In addition, new insights into immunology and genetics of lymphomas will continue to offer new therapeutic approaches, e.g. monoclonal antibodies directed against specific proteins on the surface of malignant lymphoma cells, which have already been widely applied. Also, molecular markers can be used for early detection and monitoring for relapse after or during therapy with a several-fold higher sensitivity compared to standard diagnostic techniques (Corradini et al., Leukemia 1999, 13:1691). Within the last decade numerous genes have been shown to be differentially expressed between different subtypes of NHLs. For example, CLL/SLL cases show a consistent upregulation of bcl-2 (Schena et al., Blood 1992, 79:2981). Mantle cell lymphomas are typically associated with the t(11;14)(q13;q32) translocation, leading to overexpression of bcl-1 (Raffeld et al., Blood 1991, 78;259, Seto et al., Oncogene 1992, 7;1401). The most prominent feature of follicular lymphomas is the t(14;18)(q32;q21) translocation, which is associated with a deregulation of bcl-2, a protein with anti-apoptotic properties thought to be responsible for the emergence of long-surviving memory B cells (Hockenberg et al., PNAS USA 1991, 88:6961). So far, no single marker has been shown to be sufficient for the correct diagnosis of one of the subtypes.

High-dimensional mRNA based approaches have recently been applied to Non-Hodgkin's lymphoma and other cancers. They seem to provide a better means to distinguish between different subtypes and further subclassify the disease according to the clinical outcome (Alizadeh et al., Nature. 2000 403:503-11, Hofman et al., Blood. 2001;98:787-794). However, application as a routine diagnostic tool in a clinical environment is impeded by the extreme instability of mRNA, the rapidly occurring expression changes following certain triggers (e.g.

sample collection), and, most importantly, the large amount of mRNA needed for analysis (Lipshutz, R. J. et al., Nature Genetics 21:20-24, 1999; Bowtell, D. D. L. Nature genetics suppl. 21:25-32, 1999), which often cannot be obtained from a routine biopsy.

Aberrant DNA methylation within CpG islands is common in human malignancies leading to abrogation or overexpression of a broad spectrum of genes (Jones, P.A. Cancer Res 65:2463-2467, 1996). Abnormal methylation has also been shown to occur in CpG rich regulatory elements in intronic and coding parts of genes for certain tumours (Chan, M.F., et al., Curr Top Microbiol Immunol 249:75-86,2000). Highly characteristic DNA methylation patterns could also be shown for breast cancer cell lines (Huang, T. H.-M., et al., Hum Mol Genet 8:459-470, 1999). Large-scale methylation analysis has not been applied to lymphomas so far, but alterations of the methylation of single genes have been described in several subtypes of Non-Hodgkin lymphoma, e.g. TCL1 (Yuille et al., Genes Chromosomes Cancer 2001, 30:336-41), p15 and AR (Baur et al., Blood. 1999, 94:1773-81, Martinez-Delgado et al., Leukemia. 1998 12:937-41), the androgen receptor (McDonald et al., Genes Chromosomes Cancer. 2000 28:246-57), and the MyoD1 gene (Taylor et al., Leukemia. 2001, 15:583-9).

5-methylcytosine is the most frequent covalent base modification in the DNA of eukaryotic cells. It plays a role, for example, in the regulation of the transcription, in genetic imprinting, and in tumorigenesis. Therefore, the identification of 5-methylcytosine as a component of genetic information is of considerable interest. However, 5-methylcytosine positions cannot be identified by sequencing since 5-methylcytosine has the same base pairing behaviour as cytosine. Moreover, the epigenetic information carried by 5-methylcytosine is completely lost during PCR amplification.

A relatively new and currently the most frequently used method for analysing DNA for 5-methylcytosine is based upon the specific reaction of bisulfite with cytosine which, upon subsequent alkaline hydrolysis, is converted to uracil which corresponds to thymidine in its base pairing behaviour. However, 5-methylcytosine remains unmodified under these conditions. Consequently, the original DNA is converted in such a manner that methylcytosine, which originally could not be distinguished from cytosine by its hybridisation behaviour, can now be detected as the only remaining cytosine using "normal" molecular biological techniques, for example, by amplification and hybridisation or sequencing. All of these techniques are based on base pairing which can now be fully exploited. In terms of sensitivity, the prior art is de-

fined by a method which encloses the DNA to be analysed in an agarose matrix, thus preventing the diffusion and renaturation of the DNA (bisulfite only reacts with single-stranded DNA), and which replaces all precipitation and purification steps with fast dialysis (Olek A, Oswald J, Walter J. A modified and improved method for bisulphite based cytosine methylation analysis. Nucleic Acids Res. 1996 Dec 15;24(24):5064-6). Using this method, it is possible to analyse individual cells, which illustrates the potential of the method. However, currently only individual regions of a length of up to approximately 3000 base pairs are analysed, a global analysis of cells for thousands of possible methylation events is not possible. However, this method cannot reliably analyse very small fragments from small sample quantities either. These are lost through the matrix in spite of the diffusion protection.

An overview of the further known methods of detecting 5-methylcytosine may be gathered from the following review article: Rein, T., DePamphilis, M. L., Zorbas, H., Nucleic Acids Res. 1998, 26, 2255.

To date, barring few exceptions (e.g., Zeschnigk M, Lich C, Buiting K, Doerfler W, Horsthemke B. A single-tube PCR test for the diagnosis of Angelman and Prader-Willi syndrome based on allelic methylation differences at the SNRPN locus. Eur J Hum Genet. 1997 Mar-Apr;5(2):94-8) the bisulfite technique is only used in research. Always, however, short, specific fragments of a known gene are amplified subsequent to a bisulfite treatment and either completely sequenced (Olek A, Walter J. The pre-implantation ontogeny of the H19 methylation imprint. Nat Genet. 1997 Nov;17(3):275-6) or individual cytosine positions are detected by a primer extension reaction (Gonzalgo ML, Jones PA. Rapid quantitation of methylation differences at specific sites using methylation-sensitive single nucleotide primer extension (Ms-SNuPE). Nucleic Acids Res. 1997 Jun 15;25(12):2529-31, WO 95/00669) or by enzymatic digestion (Xiong Z, Laird PW. COBRA: a sensitive and quantitative DNA methylation assay. Nucleic Acids Res. 1997 Jun 15;25(12):2532-4). In addition, detection by hybridisation has also been described (Olek et al., WO 99/28498).

Further publications dealing with the use of the bisulfite technique for methylation detection in individual genes are: Grigg G, Clark S. Sequencing 5-methylcytosine residues in genomic DNA. Bioassays. 1994 Jun;16(6):431-6, 431; Zeschnigk M, Schmitz B, Dittrich B, Buiting K, Horsthemke B, Doerfler W. Imprinted segments in the human genome: different DNA methylation patterns in the Prader-Willi/Angelman syndrome region as determined by the genomic

sequencing method. Hum Mol Genet. 1997 Mar;6(3):387-95; Feil R, Charlton J, Bird AP, Walter J, Reik W. Methylation analysis on individual chromosomes: improved protocol for bisulphite genomic sequencing. Nucleic Acids Res. 1994 Feb 25;22(4):695-6; Martin V, Ribieras S, Song-Wang X, Rio MC, Dante R. Genomic sequencing indicates a correlation between DNA hypomethylation in the 5' region of the pS2 gene and its expression in human breast cancer cell lines. Gene. 1995 May 19;157(1-2):261-4; WO 97/46705, WO 95/15373 and WO 97/45560.

An overview of the prior art in oligomer array manufacturing can be gathered from a special edition of Nature Genetics (Nature Genetics Supplement, Volume 21, January 1999), published in January 1999, and from the literature cited therein.

Fluorescently labelled probes are often used for the scanning of immobilised DNA arrays. The simple attachment of Cy3 and Cy5 dyes to the 5'-OH of the specific probe are particularly suitable for fluorescence labels. The detection of the fluorescence of the hybridised probes may be carried out, for example via a confocal microscope. Cy3 and Cy5 dyes, besides many others, are commercially available.

Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-TOF) is a very efficient development for the analysis of biomolecules (Karas M, Hillenkamp F. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal Chem. 1988 Oct 15;60(20):2299-301). An analyte is embedded in a light-absorbing matrix. The matrix is evaporated by a short laser pulse thus transporting the analyte molecule into the vapor phase in an unfragmented manner. The analyte is ionised by collisions with matrix molecules. An applied voltage accelerates the ions into a field-free flight tube. Due to their different masses, the ions are accelerated at different rates. Smaller ions reach the detector sooner than bigger ones.

MALDI-TOF spectrometry is excellently suited to the analysis of peptides and proteins. The analysis of nucleic acids is somewhat more difficult (Gut I G, Beck S. DNA and Matrix Assisted Laser Desorption Ionization Mass Spectrometry. Current Innovations and Future Trends. 1995, 1; 147-57). The sensitivity to nucleic acids is approximately 100 times worse than to peptides and decreases disproportionally with increasing fragment size. For nucleic acids having a multiply negatively charged backbone, the ionisation process via the matrix is

considerably less efficient. In MALDI-TOF spectrometry, the selection of the matrix plays an eminently important role. For the desorption of peptides, several very efficient matrixes have been found which produce a very fine crystallisation. There are now several responsive matrixes for DNA, however, the difference in sensitivity has not been reduced. The difference in sensitivity can be reduced by chemically modifying the DNA in such a manner that it becomes more similar to a peptide. Phosphorothioate nucleic acids in which the usual phosphates of the backbone are substituted with thiophosphates can be converted into a charge-neutral DNA using simple alkylation chemistry (Gut IG, Beck S. A procedure for selective DNA alkylation and detection by mass spectrometry. Nucleic Acids Res. 1995 Apr 25;23(8):1367-73). The coupling of a charge tag to this modified DNA results in an increase in sensitivity to the same level as that found for peptides. A further advantage of charge tagging is the increased stability of the analysis against impurities which make the detection of unmodified substrates considerably more difficult.

Genomic DNA is obtained from DNA of cell, tissue or other test samples using standard methods. This standard methodology is found in references such as Fritsch and Maniatis eds., Molecular Cloning: A Laboratory Manual, 1989.

The invention provide a method for the analysis of biological samples for features associated with the development of lymphoid cell proliferative disorders, characterised in that the nucleic acid of at least one member of the group comprising MDR1, CSNK2B, EGR4, AR, CDK4, RB1, CDC25A, GPIb beta, MYOD1, CDH3, MYCL1, ELK1, ABL1, APC, BCL2, CDH1, CDKN1A, CDKN1B, CDKN2a, CDKN2B, FOS, GSTP1, HIC-1, MGMT, MLH1, MOS, MYC, PTEN, RBL2, TGFBR2, TP73, CDKN1C, GSK3ß, ESR1, APAF1, BAK1, BAX and HOXA5 is/are contacted with a reagent or series of reagents capable of distinguishing between methylated and non methylated CpG dinucleotides within the genomic sequence of interest.

The present invention makes available a method for ascertaining genetic and/or epigenetic parameters of genomic DNA. The method is for use in the improved diagnosis, treatment and monitoring of lymphoid cell proliferative disorders, more specifically by enabling the improved identification of and differentiation between subclasses of said disorder and the genetic predisposition to said disorders. The invention presents improvements over the state of

the art in that it enables a highly specific classification of lymphomas, thereby allowing for improved informed treatment of patients.

In a particularly preferred embodiment the present invention makes available methods and nucleic acids that allow the differentiation between mantle cell lymphoma (hereinafter referred to as MCL), diffuse B-cell lymphoma (hereinafter referred to as DBCL), follicular lymphoma (FL), and chronic lymphocytic leukaemia, also known as small lymphocytic lymphoma (hereinafter referred to as CLL or SLL).

Furthermore, the method enables the analysis of cytosine methylations and single nucleotide polymorphisms.

In a preferred embodiment, the method comprises the following steps:

In the first step of the method the genomic DNA sample must be isolated from tissue or cellular sources. Such sources may include lymphoid tissue samples, cell lines, histological slides, body fluids, or tissue embedded in paraffin. Extraction may be by means that are standard to one skilled in the art, these include the use of detergent lysates, sonification and vortexing with glass beads. Once the nucleic acids have been extracted the genomic double stranded DNA is used in the analysis.

In a preferred embodiment the DNA may be cleaved prior to the next step of the method, this may be by any means standard in the state of the art, in particular, but not limited to, with restriction endonucleases.

In the second step of the method, the genomic DNA sample is treated in such a manner that cytosine bases which are unmethylated at the 5'-position are converted to uracil, thymine, or another base which is dissimilar to cytosine in terms of hybridisation behaviour. This will be understood as 'pretreatment' hereinafter.

The above described treatment of genomic DNA is preferably carried out with bisulfite (sulfite, disulfite) and subsequent alkaline hydrolysis which results in a conversion of non-methylated cytosine nucleobases to uracil or to another base which is dissimilar to cytosine in terms of base pairing behaviour. If bisulfite solution is used for the reaction, then an addition takes place at the non-methylated cytosine bases. Moreover, a denaturating reagent or solvent

as well as a radical interceptor must be present. A subsequent alkaline hydrolysis then gives rise to the conversion of non-methylated cytosine nucleobases to uracil. The chemically converted DNA is then used for the detection of methylated cytosines.

Fragments of the pretreated DNA are amplified, using sets of primer oligonucleotides according to SEQ ID NO: 213 to SEQ ID NO: 290, and a, preferably heat-stable, polymerase. Because of statistical and practical considerations, preferably more than ten different fragments having a length of 100 - 2000 base pairs are amplified. The amplification of several DNA segments can be carried out simultaneously in one and the same reaction vessel. Usually, the amplification is carried out by means of a polymerase chain reaction (PCR).

The method may also be enabled by the use of alternative primers, the design of such primers is obvious to one skilled in the art. These should include at least two oligonucleotides whose sequences are each reverse complementary or identical to an at least 18 base-pair long segment of the base sequences specified in the appendix (SEQ ID NO: 61 through SEQ ID NO: 212). Said primer oligonucleotides are preferably characterised in that they do not contain any CpG dinucleotides. In a particularly preferred embodiment of the method, the sequence of said primer oligonucleotides are designed so as to selectively anneal to and amplify, only the lymphoid tissue specific DNA of interest, thereby minimising the amplification of background or non relevant DNA. In the context of the present invention, background DNA is taken to mean genomic DNA which does not have a relevant tissue specific methylation pattern, in this case, the relevant tissue being lymphoid, both healthy and diseased.

According to the present invention, it is preferred that at least one primer oligonucleotide is bound to a solid phase during amplification. The different oligonucleotide and/or PNA-oligomer sequences can be arranged on a plane solid phase in the form of a rectangular or hexagonal lattice, the solid phase surface preferably being composed of silicon, glass, polystyrene, aluminium, steel, iron, copper, nickel, silver, or gold, it being possible for other materials such as nitrocellulose or plastics to be used as well.

The fragments obtained by means of the amplification can carry a directly or indirectly detectable label. Preferred are labels in the form of fluorescence labels, radionuclides, or detachable molecule fragments having a typical mass which can be detected in a mass spectrometer, it being preferred that the fragments that are produced have a single positive or negative net

charge for better detectability in the mass spectrometer. The detection may be carried out and visualised by means of matrix assisted laser desorption/ionisation mass spectrometry (MALDI) or using electron spray mass spectrometry (ESI).

The amplificates obtained in the second step of the method are subsequently hybridised to an array or a set of oligonucleotides and/or PNA probes. In this context, the hybridisation takes place in the manner described as follows. The set of probes used during the hybridisation is preferably composed of at least 10 oligonucleotides or PNA-oligomers. In the process, the amplificates serve as probes which hybridise to oligonucleotides previously bonded to a solid phase. In a particularly preferred embodiment, the oligonucleotides are taken from the group comprising SEQ ID NO: 291 to SEQ ID NO: 602. In a further preferred embodiment the oligonucleotides are taken from the group comprising SEQ ID NO: 559 to SEQ ID NO: 602. The non-hybridised fragments are subsequently removed. Said oligonucleotides contain at least one base sequence having a length of 10 nucleotides which is reverse complementary or identical to a segment of the base sequences specified in the appendix, the segment containing at least one CpG or TpG dinucleotide. In a further preferred embodiment the cytosine of the CpG dinucleotide, or in the case of TpG, the thiamine, is the 5th to 9th nucleotide from the 5'-end of the 10-mer. One oligonucleotide exists for each CpG or TpG dinucleotide.

In the fifth step of the method, the non-hybridised amplificates are removed.

In the final step of the method, the hybridised amplificates are detected. In this context, it is preferred that labels attached to the amplificates are identifiable at each position of the solid phase at which an oligonucleotide sequence is located.

According to the present invention, it is preferred that the labels of the amplificates are fluorescence labels, radionuclides, or detachable molecule fragments having a typical mass which can be detected in a mass spectrometer. The mass spectrometer is preferred for the detection of the amplificates, fragments of the amplificates or of probes which are complementary to the amplificates, it being possible for the detection to be carried out and visualised by means of matrix assisted laser desorption/ionisation mass spectrometry (MALDI) or using electron spray mass spectrometry (ESI). The produced fragments may have a single positive or negative net charge for better detectability in the mass spectrometer.

The aforementioned method is preferably used for ascertaining genetic and/or epigenetic parameters of genomic DNA.

In order to enable this method, the invention further provides the modified DNA of genes MDR1, CSNK2B, EGR4, AR, CDK4, RB1, CDC25A, GPIb beta, MYOD1, CDH3, MYCL1, ELK1, ABL1, APC, BCL2, CDH1, CDKN1A, CDKN1B, CDKN2a, CDKN2B, FOS, GSTP1, HIC-1, MGMT, MLH1, MOS, MYC, PTEN, RBL2, TGFBR2, TP73, CDKN1C, GSK3ß, ESR1, APAF1, BAK1, BAX and HOXA5 as well as oligonucleotides and/or PNA-oligomers for detecting cytosine methylations within said genes. The present invention is based on the discovery that genetic and epigenetic parameters and, in particular, the cytosine methylation patterns of genomic DNA are particularly suitable for improved diagnosis, treatment and monitoring of lymphoid cell proliferative disorders. Furthermore, the invention enables the differentiation between different subclasses of lymphomas or detection of a predisposition to lymphomas.

The nucleic acids according to the present invention can be used for the analysis of genetic and/or epigenetic parameters of genomic DNA.

This objective is achieved according to the present invention using a nucleic acid containing a sequence of at least 18 bases in length of the pretreated genomic DNA according to one of SEQ ID NO: 61 through SEQ ID NO: 212 and sequences complementary thereto.

The modified nucleic acid could heretofore not be connected with the ascertainment of disease relevant genetic and epigenetic parameters.

The object of the present invention is further achieved by an oligonucleotide or oligomer for the analysis of pretreated DNA, for detecting the genomic cytosine methylation state, said oligonucleotide containing at least one base sequence having a length of at least 10 nucleotides which hybridises to a pretreated genomic DNA according to SEQ ID NO: 61 through SEQ ID NO: 212. The oligomer probes according to the present invention constitute important and effective tools which, for the first time, make it possible to ascertain specific genetic and epigenetic parameters during the analysis of biological samples for features associated with the development of lymphoid cell proliferative disorders. Said oligonucleotides allow the improved diagnosis, treatment and monitoring of lymphoid cell proliferative disorders and

detection of the predisposition to said disorders. Furthermore, they allow the differentiation of different subclasses of lymphomas. The base sequence of the oligomers preferably contains at least one CpG or TpG dinucleotide. The probes may also exist in the form of a PNA (peptide nucleic acid) which has particularly preferred pairing properties. Particularly preferred are oligonucleotides according to the present invention in which the cytosine of the CpG dinucleotide is the 5th - 9th nucleotide from the 5'-end of the 13-mer; in the case of PNA-oligomers, it is preferred for the cytosine of the CpG dinucleotide to be the 4th - 6th nucleotide from the 5'-end of the 9-mer.

The oligomers according to the present invention are normally used in so called "sets" which contain at least one oligomer for each of the CpG dinucleotides within SEQ ID NO: 61 through SEQ ID NO: 212. Preferred is a set which contains at least one oligomer for each of the CpG dinucleotides, from SEQ ID NO: 291 through SEQ ID NO: 602. Further preferred is a set comprising SEQ ID NO: 559 to SEQ ID NO: 602.

In the case of the sets of oligonucleotides according to the present invention, it is preferred that at least one oligonucleotide is bound to a solid phase. It is further preferred that all the oligonucleotides of one set are bound to a solid phase.

The present invention moreover relates to a set of at least 10 n (oligonucleotides and/or PNA-oligomers) used for detecting the cytosine methylation state of genomic DNA using treated versions of said genomic DNA (according to SEQ ID NO: 61 through SEQ ID NO: 212 and sequences complementary thereto). These probes enable improved diagnosis, treatment and monitoring of lymphoid cell proliferative disorders progression. In particular they enable the differentiation between different sub classes of lymphoid cell proliferative disorders and the detection of a predisposition to said disorders. In a particularly preferred embodiment the set comprises SEQ ID NO: 39 to SEQ ID NO: 602.

The set of oligomers may also be used for detecting single nucleotide polymorphisms (SNPs) using pretreated genomic DNA according to one of SEQ ID NO: 61 through SEQ ID NO: 212.

According to the present invention, it is preferred that an arrangement of different oligonucleotides and/or PNA-oligomers (a so-called "array") made available by the present invention

is present in a manner that it is likewise bound to a solid phase. This array of different oligonucleotide- and/or PNA-oligomer sequences can be characterised in that it is arranged on the solid phase in the form of a rectangular or hexagonal lattice. The solid phase surface is preferably composed of silicon, glass, polystyrene, aluminium, steel, iron, copper, nickel, silver, or gold. However, nitrocellulose as well as plastics such as nylon which can exist in the form of pellets or also as resin matrices are suitable alternatives.

Therefore, a further subject matter of the present invention is a method for manufacturing an array fixed to a carrier material for the improved diagnosis, treatment and monitoring of lymphoid cell proliferative disorders, the differentiation between different subclasses of lymphomas and/or detection of the predisposition to lymphoid cell proliferative disorders. In said method at least one oligomer according to the present invention is coupled to a solid phase. Methods for manufacturing such arrays are known, for example, from US Patent 5,744,305 by means of solid-phase chemistry and photolabile protecting groups.

A further subject matter of the present invention relates to a DNA chip for the improved diagnosis, treatment and monitoring of lymphoid cell proliferative disorders. Furthermore the DNA chip enables detection of the predisposition to lymphoid cell proliferative disorders and the differentiation between different subclasses of lymphomas. The DNA chip contains at least one nucleic acid according to the present invention. DNA chips are known, for example, in US Patent 5,837,832.

Moreover, a subject matter of the present invention is a kit which may be composed, for example, of a bisulfite-containing reagent, a set of primer oligonucleotides containing at least two oligonucleotides whose sequences in each case correspond or are complementary to a 18 base long segment of the base sequences specified in the appendix (SEQ ID NO: 61 through SEQ ID NO: 212), oligonucleotides and/or PNA-oligomers as well as instructions for carrying out and evaluating the described method. However, a kit along the lines of the present invention can also contain only part of the aforementioned components.

The oligomers according to the present invention or arrays thereof as well as a kit according to the present invention are intended to be used for the improved diagnosis, treatment and monitoring of lymphoid cell proliferative disorders. Furthermore the use of said inventions extends to the differentiation between different subclasses of lymphomas and detection of the

predisposition to lymphoid cell proliferative disorders. According to the present invention, the method is preferably used for the analysis of important genetic and/or epigenetic parameters within genomic DNA, in particular for use in improved diagnosis, treatment and monitoring of lymphoid cell proliferative disorders, detection of the predisposition to said disorders and the differentiation between subclasses of said disorders.

The methods according to the present invention are used, for example, for improved diagnosis, treatment and monitoring of lymphoid cell proliferative disorders progression, detection of the predisposition to said disorders and the differentiation between subclasses of said disorders.

A further embodiment of the invention is a method for the analysis of the methylation status of genomic DNA without the need for pre-treatment. In the first step of the method the genomic DNA sample must be isolated from tissue or cellular sources. Such sources may include cell lines, histological slides, body fluids, or tissue embedded in paraffin. Extraction may be by means that are standard to one skilled in the art, these include the use of detergent lysates, sonification and vortexing with glass beads. Once the nucleic acids have been extracted the genomic double stranded DNA is used in the analysis.

In a preferred embodiment the DNA may be cleaved prior to the treatment, this may be any means standard in the state of the art, in particular with restriction endonucleases. In the second step, the DNA is then digested with one or more methylation sensitive restriction enzymes. The digestion is carried out such that hydrolysis of the DNA at the restriction site is informative of the methylation status of a specific CpG dinucleotide.

In the third step the restriction fragments are amplified. In a preferred embodiment this is carried out using a polymerase chain reaction.

In the final step the amplificates are detected. The detection may be by any means standard in the art, for example, but not limited to, gel electrophoresis analysis, hybridisation analysis, incorporation of detectable tags within the PCR products, DNA array analysis, MALDI or ESI analysis.

The present invention moreover relates to the diagnosis and/or prognosis of events which are disadvantageous or relevant to patients or individuals in which important genetic and/or epigenetic parameters within genomic DNA, said parameters obtained by means of the present invention may be compared to another set of genetic and/or epigenetic parameters, the differences serving as the basis for the diagnosis and/or prognosis of events which are disadvantageous or relevant to patients or individuals.

The genes that form the basis of the present invention can be used to form a "gene panel", i. e. a collection that comprises the particular genes of the present invention and/or their respective informative methylation sites. The formation of gene panels enables a fast and specific analysis of the disorders related therewith. The gene panels as used and described in the present invention can be employed for the diagnosis, treatment and monitoring and the analysis of a predisposition for the disorders described herein with surprisingly high efficiencies.

The use of a plurality of CpG-sites from a divers array of genes that specifically regulate cell proliferative disorders, in addition allows for a relatively high degree of sensitivity and specificity in comparison with tools that comprise singular gene-diagnosis- and gene-determination-instruments. Furthermore, the panel as described herein, in contrast to other methods available, can be designed to fit to a more specific use in the analyses of multiple disorders that are all specifically caused by cell proliferative disorders.

In the context of the present invention the term "hybridisation" is to be understood as a bond of an oligonucleotide to a completely complementary sequence along the lines of the Watson-Crick base pairings in the sample DNA, forming a duplex structure.

In the context of the present invention, "genetic parameters" are mutations and polymorphisms of genomic DNA and sequences further required for their regulation. To be designated as mutations are, in particular, insertions, deletions, point mutations, inversions and polymorphisms and, particularly preferred, SNPs (single nucleotide polymorphisms).

In the context of the present invention, "epigenetic parameters" are, in particular, cytosine methylations and further modifications of DNA bases of genomic DNA and sequences further required for their regulation. Further epigenetic parameters include, for example, the acetyla-

tion of histones which, cannot be directly analysed using the described method but which, in turn, correlates with the DNA methylation.

In the following, the present invention will be explained in more detail on the basis of the figures, sequences and examples without being limited thereto. In the attached figures,

Figure 1 shows the source of all samples that were used in the analyses described in examples 1 and 2. Column "1" indicates the sample number, which is applicable also for figures 2 to 7. Column "2" shows the diagnosis of the disorder, wherein MCL is mantle cell-lymphoma, FL means follicular lymphoma and CLL/SLL means chronic lymphocytic leukaemia/small lymphocytic lymphoma, and DLBCL means diffuse large B-cell-lymphoma. Column "3" indicates the age of the patient, from which the sample was obtained. Column "4" indicates the gender age of the patient, from which the sample was obtained.

Figure 2 shows the differentiation of MCL, DLBCL and CLL/SLL from FL I and II, according to example 2. The markers on the left side of the plot are gene- and CpG-identifiers, these correspond to the ones of table 3. The markers on the right side indicate the significance (p-value, T-test) of the difference between the means of the two groups. Each row correspond to a single CpG and each column to the methylation grade of a sample. The CpGs are sorted according to their contribution for the distinctiveness of the differential diagnosis of the two lymphoma, with increasing contribution from top to bottom. Black indicates a complete methylation of a particular CpG-position, white indicates no methylation at the particular position, with graduations of methylation which are indicated in grey, from light (small portion of methylation) to dark (large portion of methylation).

<u>Figure 3</u> shows the differentiation of MCL, DLBCL and CLL/SLL from Fl I and II (only male samples), according to example 2. The markers on the left side of the plot are gene- and CpG-identifiers, these correspond to the ones of table 4. The markers on the right side indicate the significance (p-value, T-test) of the difference between the means of the two groups. Each row correspond to a single CpG and each column to the methylation grade of a sample. The CpGs are sorted according to their contribution for the distinctiveness of the differential diagnosis of the two lymphoma, with increasing contribution from top to bottom. Black indicates a complete methylation of a particular CpG-position, white indicates no methylation at the particular position, with graduations of methylation which are indicated in grey, from light (small portion of methylation) to dark (large portion of methylation).

<u>Figure 4</u> shows the differentiation of MCL from follicular lymphoma, according to example 2. The markers on the left side of the plot are gene- and CpG-identifiers, these correspond to the ones of table 5. The markers on the right side indicate the significance (p-value, T-test) of the difference between the means of the two groups. Each row correspond to a single CpG and each column to the methylation grade of a sample. The CpGs are sorted according to their contribution for the distinctiveness of the differential diagnosis of the two lymphoma, with increasing contribution from top to bottom. Black indicates a complete methylation of a particular CpG-position, white indicates no methylation at the particular position, with graduations of methylation which are indicated in grey, from light (small portion of methylation) to dark (large portion of methylation).

Figure 5 shows the differentiation of MCL from from follicular lymphoma (only male samples), according to example 2. The markers on the left side of the plot are gene- and CpG-identifiers, these correspond to the ones of table 6. The markers on the right side indicate the significance (p-value, T-test) of the difference between the means of the two groups. Each row correspond to a single CpG and each column to the methylation grade of a sample. The CpGs are sorted according to their contribution for the distinctiveness of the differential diagnosis of the two lymphoma, with increasing contribution from top to bottom. Black indicates a complete methylation of a particular CpG-position, white indicates no methylation at the particular position, with graduations of methylation which are indicated in grey, from light (small portion of methylation) to dark (large portion of methylation).

<u>Figure 6</u> shows the differentiation of FL from CLL/SLL, according to example 2: The markers on the left side of the plot are gene- and CpG-identifiers, these correspond to the ones of table 7. The markers on the right side indicate the significance (p-value, T-test) of the difference between the means of the two groups. Each row correspond to a single CpG and each column to the methylation grade of a sample. The CpGs are sorted according to their contribution for the distinctiveness of the differential diagnosis of the two lymphoma, with increasing contribution from top to bottom. Black indicates a complete methylation of a particular CpG-position, white indicates no methylation at the particular position, with graduations of methylation which are indicated in grey, from light (small portion of methylation) to dark (large portion of methylation).

Figure 7 shows the differentiation of FL from CLL/SLL (only male samples), according to example 2. The markers on the left side of the plot are gene- and CpG-identifiers, these correspond to the ones of table 8. The markers on the right side indicate the significance (p-value, T-test) of the difference between the means of the two groups. Each row correspond to a single CpG and each column to the methylation grade of a sample. The CpGs are sorted according to their contribution for the distinctiveness of the differential diagnosis of the two lymphoma, with increasing contribution from top to bottom. Black indicates a complete methylation of a particular CpG-position, white indicates no methylation at the particular position, with graduations of methylation which are indicated in grey, from light (small portion of methylation) to dark (large portion of methylation).

SEQ ID NO: 1 to SEQ ID NO: 38 represent 5' and/or regulatory regions of the genomic DNA of genes MDR1, CSNK2B, EGR4, AR, CDK4, RB1, CDC25A, GPIb beta, MYOD1, CDH3, MYCL1, ELK1, ABL1, APC, BCL2, CDH1, CDKN1A, CDKN1B, CDKN2a, CDKN2B, FOS, GSTP1, HIC-1, MGMT, MLH1, MOS, MYC, PTEN, RBL2, TGFBR2, TP73, CDKN1C, GSK3ß, ESR1, APAF1, BAK1, BAX and HOXA5. These sequences are derived from Genbank and will be taken to include all minor variations of the sequence material which are currently unforeseen, for example, but not limited to, minor deletions and SNPs.

SEQ ID NO: 61 to SEQ ID NO: 212 exhibit the pretreated sequence of DNA derived from genes MDR1, CSNK2B, EGR4, AR, CDK4, RB1, CDC25A, GPIb beta, MYOD1, CDH3, MYCL1, ELK1, ABL1, APC, BCL2, CDH1, CDKN1A, CDKN1B, CDKN2a, CDKN2B, FOS, GSTP1, HIC-1, MGMT, MLH1, MOS, MYC, PTEN, RBL2, TGFBR2, TP73, CDKN1C, GSK3ß, ESR1, APAF1, BAK1, BAX and HOXA5. These sequences will be taken to include all minor variations of the sequence material which are currently unforeseen, for example, but not limited to, minor deletions and SNPs.

SEQ ID NO: 213 to SEQ ID NO:290 exhibit the sequence of primer oligonucleotides for the amplification of pretreated DNA according to Sequence IDs 61 to 212.

SEQ ID NO: 291 to SEQ ID NO: 602 exhibit the sequence of oligomers which are useful for the analysis of CpG positions within genomic DNA according to SEQ ID NO: 1 to SEQ ID NO: 38.

SEQ ID NO: 559 to SEQ ID NO: 602 exhibit the sequence of oligomers which are useful for the analysis of CpG positions within genomic DNA according to SEQ ID NO: 1 to SEQ ID NO: 38.

Examples 1 and 2: Digital Phenotype

In the following examples, multiplex PCR was carried out on samples from patients with different subclasses of lymphomas (see Figure 1 for further details). Each sample was treated in the manner described below in Example 1 in order to deduce the methylation status of CpG positions, the CpG methylation information for each sample was collated and then used in an analysis, as detailed in Example 2. An alternative example for the analysis of the CpG-methylation status is furthermore described in example 3.

Example 1

In the first step the genomic DNA was isolated from the cell samples using the Wizzard kit from (Promega). The isolated genomic DNA from the samples are treated using a bisulfite solution (hydrogen sulfite, disulfite). The treatment is such that all non methylated cytosines within the sample are converted to thiamine, conversely 5-methylated cytosines within the sample remain unmodified. The treated nucleic acids were then amplified using multiplex PCRs, amplifying 8 fragments per reaction with Cy5 fluorescently labelled primers. PCR primers used are described in Table 1. PCR conditions were as follows.

Reaction solution: 10 ng bisulfite treated DNA 3,5 mM MgCl2 400 µM dNTPs 2 pmol each primer 1 U Hot Star Taq (Qiagen)

Forty cycles were carried out as follows. Denaturation at 95°C for 15 min, followed by annealing at 55°C for 45 sec., primer elongation at 65°C for 2 min. A final elongation at 65°C was carried out for 10 min. All PCR products from each individual sample were then hybridised to glass slides carrying a pair of immobilised oligonucleotides for each CpG position under analysis. Each of these detection oligonucleotides was designed to hybridise to the bisulphite converted sequence around one CpG site which was either originally unmethylated (TG) or methylated (CG). See Table 2 for further details of all hybridisation oligonucleotides

used (both informative and non-informative) Hybridisation conditions were selected to allow the detection of the single nucleotide differences between the TG and CG variants.

 $5~\mu l$ volume of each multiplex PCR product was diluted in 10~x Ssarc buffer (10~x Ssarc:230 ml 20~x SSC, 180~m l sodium lauryl sarcosinate solution 20%, dilute to 1000~m l with dH2O). The reaction mixture was then hybridised to the detection oligonucleotides as follows. Denaturation at $95^{\circ}C$, cooling down to $10~^{\circ}C$, hybridisation at $42^{\circ}C$ overnight followed by washing with 10~x Ssarc and dH_2O at $42^{\circ}C$.

Fluorescent signals from each hybridised oligonucleotide were detected using genepix® scanner and software. Ratios for the two signals (from the CG oligonucleotide and the TG oligonucleotide used to analyse each CpG position) were calculated based on comparison of intensity of the fluorescent signals.

Example 2

The information is then sorted into a ranked matrix (as shown in Figures 2 to 7) according to CpG methylation differences between the two classes of tissues, using an algorithim. The most significant CpG positions are at the bottom of the matrix with significance decreasing towards the top. Black indicates total methylation at a given CpG position, white represents no methylation at the particular position, with degrees of methylation represented in gray, from light (low proportion of methylation) to dark (high proportion of methylation). Each row represents one specific CpG position within a gene and each column shows the methylation profile for the different CpGs for one sample. On the left side a CpG and gene identifier is shown this may be cross referenced with the accompanying table (Tables 3 to 8) in order to ascertain the gene in question and the detection oligomer used. On the right side p values for the individual CpG positions are shown. The p values are the probabilities that the observed distribution occurred by chance in the data set.

For selected distinctions, we trained a learning algorithm (support vector machine, SVM). The SVM (as discussed by F. Model, P. Adorjan, A. Olek, C. Piepenbrock, Feature selection for DNA methylation based cancer classification. Bioinformatics. 2001 Jun; 17 Suppl 1:S157-64) constructs an optimal discriminant between two classes of given training samples. In this case each sample is described by the methylation patterns (CG/TG ratios) at the investigated CpG sites. The SVM was trained on a subset of samples of each class, which were presented with

the diagnosis attached. Independent test samples, which were not shown to the SVM before were then presented to evaluate, if the diagnosis can be predicted correctly based on the predictor created in the training round. This procedure was repeated several times using different partitions of the samples, a method called crossvalidation. Please note that all rounds are performed without using any knowledge obtained in the previous runs. The number of correct classifications was averaged over all runs, which gives a good estimate of our test accuracy (percent of correct classified samples over all rounds).

MCL, DLBCL & CLL/SLL compared to FL I and II.(Figures 2 and 3)

In figure 2, the first group is composed of 42 samples of MCL, DLBCL & CLL/SLL from both sexes, which are compared to 38 samples of FL I and II. The p-value shows a clear discrimination between the two groups, 9 CpG positions from 7 distinct genes allow discrimination between the 2 groups (p<0.05). The crossvalidation accuracy performed by a SVM is calculated as 75.8% with a standard deviation of 2.8%. The significant genes and detection oligonucleotides are shown below in Table 3.

The analysis may be refined by performing the comparison between members of the same sex. The comparison of male samples only (Figure 3) increased the accuracy to 80.4% with a standard deviation of 2.9%. The significant genes and detection oligonucleotides are shown in Table 4.

MCL compared to follicular lymphoma. (Figures 4 and 5)

Comparison of male MCL samples to male and female follicular lymphoma samples. The analysis allowed for a discrimination between the 2 classes with an accuracy of 92%, with a standard deviation of 2.4%. The significant genes and detection oligonucleotides are shown in Table 5.

Comparison of male samples only from FL II identified informative CpG positions in 9 genes. The crossvalidation accuracy is 86.5%, with a standard deviation of 3.7%. The decreased accuracy is not surprising in light of the reduced sample size use for training the classifier. The significant genes and detection oligonucleotides are shown in Table 6.

FL compared to CLL/SLL (Figures 6 and 7)

Comparison of MCL to CLL/SLL, male samples only allowed a discrimination between the two groups with a classification accuracy of 91% with a standard deviation of 2.3%. The significant genes and detection oligonucleotides are shown in Tables 7 and 8.

Example 3

Identification of the methylation status of a CpG site within the gene CDKN1C. The following example illustrates an alternative manner of CpG methylation status analysis which may be used in place of the method illustrated in Example 1 to provide data in an analysis as illustrated in Example 2.

A fragment of the gene CDKN1C (Seq ID NO: 32) was PCR amplified using primers CATTTGGGGAGGCAGATA (Seq ID NO: xx) and TGTCCTTGAGAGGTGCGA (Seq ID NO: yy). The resultant fragment (262 bp in length) contained an informative CpG at position 63. The amplificate DNA was digested with the restriction endonuclease *EaeI*, recogniton site YGGCCR. Hydrolysis by said endonuclease is blocked by methylation of the CpG at position 106 of the amplificate. The digest was used as a control.

Genomic DNA was isolated from lymphoma tissues using the wizzard® DNA isolation kit (Promega). Each sample was digested using *EaeI* according to manufacturer's recommendations (New England Biolabs).

10 of each genomic digest was then amplified using PCR primers CATTTGGGGAGGCAGATA (Seq ID NO: zz) and TGTCCTTGAGAGGTGCGA (Seq ID NO: aa). The PCR reactions were performed using a thermocycler (Eppendorf GmbH) using 10 ng of DNA, 6 pmole of each primer, 200 µM of each dNTP, 1.5 mM MgCl2 and 1 U of HotstartTaq (Qiagen AG). The other conditions were as recommended by the Taq polymerase manufacturer. Using the above mentioned primers, gene fragments were amplified by PCR performing a first denaturation step for 14 min at 96 °C, followed by 30 - 45 cycles (step 2: 60 sec at 96°C, step 3: 45 sec at 52 °C, step 4: 75 sec at 72 °C) and a subsequent final elongation of 10 min at 72 °C. The presence of PCR products was analysed by agarose gel electrophoresis.

PCR products were detectable with *EaeI* hydrolysed DNA isolated from upmethylated tissue, when step 2 to step 4 of the cycle program were repeated 34, 37, 39, 42 and 45 fold. In con-

trast significant levels of PCR products were only detectable with *EaeI* hydrolysed DNA isolated from downmethylated (and the control sample) when step 2 to step 4 of the cycle program were repeated 42 and 45 fold. These results were combined in an analysis as illustrated in Example 3 with other members of a gene panel for the analysis of lymphoma tissues.

<u>Tables</u>
<u>Table 1: PCR primers and products</u>

No:	Gene:	Primer:	Primer	Size:
			type:	
1	EGR4 (SEQ ID NO: 3)	AGGGGGATTGAGTGTTAAGT (SEQ ID NO: 214) CCCAAACATAAACACAAAAT	start	293
		(SEQ ID NO: 213)	biop	
2	GPIb beta (SEQ ID NO: 8)	GGTGATAGGAGAATAATGTTGG (SEQ ID NO: 215) TCTCCCAACTACAACCAAAC (SEQ ID NO: 216)	start stop	379
3	CDKN1B (SEQ ID NO: 18)	GTGGGGAGGTAGTTGAAGA (SEQ ID NO: 217) ATACACCCCTAACCCAAAAT (SEQ ID NO: 218)	start stop	478
4	CSNK2B (SEQ ID NO: 2)	GGGGAAATGGAGAAGTGTAA (SEQ ID NO: 219) CTACCAATCCCAAAATAACC (SEQ ID NO: 220)	start stop	524
5	TP73 (SEQ ID NO: 31)	AGTAAATAGTGGGTGAGTTATGAA (SEQ ID NO: 222) GAAAAACCTCTAAAAACTACTCTCC (SEQ ID NO: 221)	start stop	607
6	RB1 (SEQ ID NO: 6)	TTTAAGTTTGTTTTTGTTTTGGT (SEQ ID NO: 223) TCCTACTCTAAATCCTCCTCAA (SEQ ID NO: 224)	start stop	718
7	CDK4 (SEQ ID NO: 5)	GGTAGTTGGTTATATGGTGAGG (SEQ ID NO: 225) TCACACTCTTAAAAACCACAAAA (SEQ ID NO: 226)	start stop	748
8	ELK1 (SEQ ID NO: 12)	AAGTGTTTTAGTTTTTAATGGGTA (SEQ ID NO: 22) CAAACCCAAAACTCACCTAT (SEQ ID NO: 228)	start stop	966
9	ABL1 (SEQ ID NO: 13)	GTTAGGAGGGGGTTAAGG (SEQ ID NO: 229) CCAACTTCAAACAAATCTCC (SEQ ID NO: 230)	start stop	291
10	FOS (SEQ ID NO: 21)	TTTTTGGGGTTTAGTTTAGAAT (SEQ ID NO: 231) AACCTTCATCCCCTAACCT	start stop	308

,		(SEQ ID NO: 232)		
11	MOS (SEQ ID NO: 26)	ACCCTACAACAATCCCTCA (SEQ ID NO: 233) TGGTTTTTAGGTTATTGGATTT (SEQ ID NO: 234)	start stop	343
12	APC (SEQ ID NO: 14)	AGGAAGTATTGAAGATGAAGTTATG (SEQ ID NO: 235) TTCCAATAAAACAATAAACTC (SEQ ID NO: 236)	start stop	
13	MOS (SEQ ID NO: 26)	TGATTGGGAGTAGGTGTGTT (SEQ ID NO: 237) CAAATCTTCCAACTTCTCAAA (SEQ ID NO: 238)	start stop	523
14	CDH3 (SEQ ID NO: 10)	GTTTAGAAGTTTAAGATTAG (SEQ ID	start stop	611
15	ESR1 (SEQ ID NO: 34)	AGGGGGAATTAAATAGAAAGAG (SEQ ID NO: 241) CAATAAAACCATCCCAAATACT (SEQ ID NO: 242)	start stop	662
16	BAX (SEQ ID NO: 37)	AAATAAATAGAAAAGTAGGTTTGGC (SEQ ID NO: 243) TTCTACCCCTCAATACTTAAAAA (SEQ ID NO: 244)	start stop	716
17	HIC-1 (SEQ ID NO: 23)	· · · · · · · · · · · · · · · · · · ·	start stop	280
18	CDKN1C (SEQ ID NO: 32)	GGGGAGGTAGATATTTGGATAA (SEQ ID NO: 247) AACTACACCATTTATATTCCCAC (SEQ ID NO: 248)	start stop	300
19	PTEN (SEQ ID NO: 28)	TTTTAGGTAGTTATATTGGGTATGTT (SEQ ID NO: 249) TCAACTCTCAAACTTCCATCA (SEQ ID NO: 250)	start stop	346
20	AR (SEQ ID NO: 4)		start stop	460
21	MLH1 (SEQ ID NO: 25)		start stop	545
22	MGMT (SEQ ID NO: 24)		start stop	636
23	APAF1 (SEQ ID NO: 35)	AGATATGTTTGGAGATTTTAGGA (SEQ ID NO: 257) AACTCCCCACCTCTAATTCTAT (SEQ ID NO: 258)	start stop	674

24	MYOD1 (SEQ ID NO: 9)	ATTAGGGGTATAGAGGAGTATTGA (SEQ ID NO: 259)	start stop	883
		CTTACAAACCCACAATAAACAA (SEQ ID NO: 260)		
25	RBL2 (SEQ ID NO: 29)	GAAAATGGGTGTGTGTGG (SEQ ID NO: 261) TACAAATAAAAACAAATCCCCT (SEQ ID NO: 262)	start stop	112
26	GSK3ß (SEQ ID NO: 33)	TAAGTGATAAAGGAAGGAAGGA (SEQ ID NO: 263) CCTTCAAACCCCAAACAA (SEQ ID NO: 264)	~	243
27	GSTP1 (SEQ ID NO: 22)	ATTTGGGAAAGAGGGAAAG (SEQ ID NO: 265) TAAAAACTCTAAACCCCATCC (SEQ II NO: 266)	start stop	300
28	CDKN1A (SEQ ID NO: 17)	GGATTAGTGGGAATAGAGGTG (SEQ ID NO: 267) AAACCCAAACTCCTAACTACC (SEQ II NO: 268)	start stop	408
29	CDH1 (SEQ ID NO: 16)	CAAATAAACCCTCAACCAATC (SEQ ID NO: 269) TGGAGGGGGTAGGAAAGT (SEQ ID NO: 270)	start stop	474
30	MDR1 (SEQ ID NO: 1)	TAAGTATGTTGAAGAAAGATTATTGT AG (SEQ ID NO: 272) TAAAAACTATCCCATAATAACTCCCA AC (SEQ ID NO: 271)	start stop	633
31	CDKN2B (SEQ ID NO: 20)	GGTTGGTTGAAGGAATAGAAAT (SEQ ID NO: 273) CCCACTAAACATACCCTTATTC (SEQ ID NO: 274)	start stop	708
32	CDKN2a (SEQ ID NO: 19)	GGGGTTGGTTGGTTATTAGA (SEQ ID NO: 275) AACCCTCTACCCACCTAAAT (SEQ ID NO: 276)	start stop	256
33	TGFBR2 (SEQ ID NO: 30)	GTAATTTGAAGAAAGTTGAGGG (SEQ ID NO: 277) CCAACAACTAAACAAAACCTCT (SEQ ID NO: 278)	start stop	296
34	HOXA5 (SEQ ID NO: 38)	AAACCCCAAACAACCTCTAT (SEQ ID NO: 280) GAAGGGGGAAAGTTATTTAGTTA (SEQ ID NO: 279)	start stop	392
35	CDC25A (SEQ ID NO: 7)	TTGGGAGTTTTTATTGATTTTT (SEQ ID NO: 281) ACAACCTAAAAATTAAATCCAAA (SEQ ID NO: 282)	start stop	445
36	MYCL1 (SEQ ID NO: 11)	AGGTTTGGGTTATTGAGTTT (SEQ ID NO: 283) CATTATTTCCTAACTACCTTATATCTC (SEQ ID NO: 284)	start stop	491
37		AATTAGGGATGGGAAAAGTAGT (SEQ ID NO: 285)	start stop	558

		AAACATAACAAAATCAAATCCC (SEQ ID NO: 286)		
38	BCL2 (SEQ ID NO: 15)	GTATTTTATGTTAAGGGGGAAA (SEQ ID NO: 287) AAAAACCACAATCCTCCC (SEQ ID NO: 288)		640
39	MYC (SEQ ID NO: 27)	AGAGGGAGTAAAAGAAAATGGT (SEQ ID NO: 289) CCAAATAAACAAAATAACCTCC (SEQ ID NO: 290)	start stop	712

Table 2: Hybridisation oligonucleotides

No:	Gene	Oligo:
,		
1	MDR1 (SEQ ID NO: 1)	TTGGTGGTCGTTTTAAGG (SEQ ID NO: 583)
2 .	MDR1 (SEQ ID NO: 1)	TTGGTGGTTGTTTTAAGG (SEQ ID NO: 584)
3	MDR1 (SEQ ID NO: 1)	TTGAAAGACGTGTTTATA (SEQ ID NO: 291)
4	MDR1 (SEQ ID NO: 1)	TTGAAAGATGTGTTTATA (SEQ ID NO: 292)
5	MDR1 (SEQ ID NO: 1)	AGGTGTAACGGAAGTTAG (SEQ ID NO: 293)
6	MDR1 (SEQ ID NO: 1)	AGGTGTAATGGAAGTTAG (SEQ ID NO: 294)
7	MDR1 (SEQ ID NO: 1)	TAGTTTTCGAGGAATTA (SEQ ID NO: 295)
8	MDR1 (SEQ ID NO: 1)	TAGTTTTTGAGGAATTA (SEQ ID NO: 296)
9	CSNK2B (SEQ ID NO: 2)	AGGAGTTTCGGAGGAAAT (SEQ ID NO: 297)
10	CSNK2B (SEQ ID NO: 2)	AGGAGTTTTGGAGGAAAT (SEQ ID NO: 298)
11	CSNK2B (SEQ ID NO: 2)	GAGAGTTGCGGAAAGAGA (SEQ ID NO: 299)
12	CSNK2B (SEQ ID NO: 2)	GAGAGTTGTGGAAAGAGA (SEQ ID NO: 300)
13	CSNK2B (SEQ ID NO: 2)	GGGTTTTCGTGATAGT (SEQ ID NO: 301)
14	CSNK2B (SEQ ID NO: 2)	GGGTTTTTGTGATAGT (SEQ ID NO: 302)
15	CSNK2B (SEQ ID NO: 2)	TAGGTTAGCGTATTGGGA (SEQ ID NO: 303)
16	CSNK2B (SEQ ID NO: 2)	TAGGTTAGTGTATTGGGA (SEQ ID NO: 304)
17	EGR4 (SEQ ID NO: 3)	GTGGGAAGCGTATTTATC (SEQ ID NO: 305)

18	EGR4 (SEQ ID NO: 3)	GTGGGAAGTGTATTTATC (SEQ ID NO: 306)
19	EGR4 (SEQ ID NO: 3)	AATAATAACGTTATAGTT (SEQ ID NO: 307)
20	EGR4 (SEQ ID NO: 3)	AATAATAATGTTATAGTT (SEQ ID NO: 308)
21	EGR4 (SEQ ID NO: 3)	TTATAGTTCGAGTTTTTT (SEQ ID NO: 309)
22	EGR4 (SEQ ID NO: 3)	TTATAGTTTGAGTTTTTT (SEQ ID NO: 310)
23	EGR4 (SEQ ID NO: 3)	GGAGTTTTCGGTATATAT (SEQ ID NO: 311)
24	EGR4 (SEQ ID NO: 3)	GGAGTTTTTGGTATATAT (SEQ ID NO: 312)
25	4)	TGTTATTTCGAGAGAGGT (SEQ ID NO: 313)
26	4)	TGTTATTTTGAGAGAGGT (SEQ ID NO: 314)
27	4)	AGAGGTTGCGTTTTAGAG (SEQ ID NO: 569)
28	4)	AGAGGTTGTGTTTTAGAG (SEQ ID NO: 570)
29	AR (SEQ ID NO: 4)	GTAGTATTCGAAGGTAGT (SEQ ID NO: 315)
30	4)	GTAGTATTTGAAGGTAGT (SEQ ID NO: 316)
31	AR (SEQ ID NO: 4)	GGAGGTTTCGGGGGTTTT (SEQ ID NO: 317)
32	AR (SEQ ID NO: 4)	GGAGGTTTTGGGGGTTTT (SEQ ID NO: 318)
33	CDK4 (SEQ ID NO: 5)	GTATGGGGTCGTAGGAAT (SEQ ID NO: 319)
34	CDK4 (SEQ ID NO: 5)	GTATGGGGTTGTAGGAAT (SEQ ID NO: 320)
35	CDK4 (SEQ ID NO: 5)	GGAAGGGTCGTTTAAGGG (SEQ ID NO: 563)
36	CDK4 (SEQ ID NO: 5)	GGAAGGGTTGTTTAAGGG (SEQ ID NO: 564)
37	CDK4 (SEQ ID NO: 5)	GGGTTGGCGTGAGGTA (SEQ ID NO: 321)
38	CDK4 (SEQ ID NO: 5)	GGGTTGGTGAGGTA (SEQ ID NO: 322)
39	CDK4 (SEQ ID NO: 5)	AGGATTTTCGATGTAAGG (SEQ ID NO: 323)
40		AGGATTTTTGATGTAAGG (SEQ ID NO: 324)
41	CDK4 (SEQ ID NO: 5)	GGGTTTTACGTGGTTGGA (SEQ ID NO: 325)
12		GGGTTTTATGTGGTTGGA (SEQ ID NO: 326)
13	· -	GAGTTTAACGTAGTAAGG (SEQ ID NO: 327)

	NO: 26)	
44	MOS (SEQ ID	GAGTTTAATGTAGTAAGG (SEQ ID NO: 328)
	NO: 26)	
45	MOS (SEQ ID NO: 26)	TATGGAGTTCGGTGGTAA (SEQ ID NO: 571)
46	MOS (SEQ ID NO: 26)	TATGGAGTTTGGTGGTAA (SEQ ID NO: 572)
47	MOS (SEQ ID NO: 26)	TTTATTGTCGTATTGGAG (SEQ ID NO: 595)
48	MOS (SEQ ID NO: 26)	TTTATTGTTGTATTGGAG (SEQ ID NO: 596)
49	MOS (SEQ ID NO: 26)	GTTGTGAACGGTTTGTTT (SEQ ID NO: 329)
50	MOS (SEQ ID NO: 26)	GTTGTGAATGGTTTGTTT (SEQ ID NO: 330)
51	RB1 (SEQ ID NO: 6)	TTAGATTTCGGGATAGGG (SEQ ID NO: 331)
52	RB1 (SEQ ID NO: 6)	TTAGATTTTGGGATAGGG (SEQ ID NO: 332)
53	RB1 (SEQ ID NO: 6)	TATAGTTTCGTTAAGTGT (SEQ ID NO: 333)
54	RB1 (SEQ ID NO: 6)	TATAGTTTGTTAAGTGT (SEQ ID NO: 334)
55	RB1 (SEQ ID NO: 6)	GTGTATTTCGGTTTGGAG (SEQ ID NO: 601)
56	RB1 (SEQ ID NO: 6)	GTGTATTTTGGTTTGGAG (SEQ ID NO: 602)
57	RB1 (SEQ ID NO: 6)	TTGGAAGGCGTTTGGATT (SEQ ID NO: 335)
58	RB1 (SEQ ID NO: 6)	TTGGAAGGTGTTTGGATT (SEQ ID NO: 336)
59		GTGTAGGTCGGTTTGGTT (SEQ ID NO: 337)
60		GTGTAGGTTGGTT (SEQ ID NO: 338)
61		TTGTTATTCGGAGTTGGG (SEQ ID NO: 339)
62		TTGTTATTTGGAGTTGGG (SEQ ID NO: 340)
63		GGAGAATAGCGAAGATAG (SEQ ID NO: 341)
64		GGAGAATAGTGAAGATAG (SEQ ID NO: 342)
65		GAAAGGTCGGTTTGGT (SEQ ID NO: 343)
66		GAAAGGTTGGTTTGGT (SEQ ID NO: 344)
67	GPIb beta (SEQ ID NO: 8)	TTTGAGAGCGGGTGGGAG (SEQ ID NO: 579)
68	GPIb beta (SEQ ID NO: 8)	TTTGAGAGTGGGTGGGAG (SEQ ID NO: 580)

		29
69	GPIb beta (SEQ ID NO: 8)	GTGGGAGCGGAAGTTTGA (SEQ ID NO: 561)
70	GPIb beta (SEQ ID NO: 8)	GTGGGAGTGGAAGTTTGA (SEQ ID NO: 562)
71	GPIb beta (SEQ ID NO: 8)	GGTTAGGTCGTAGTATTG (SEQ ID NO: 345)
72	GPIb beta (SEQ ID NO: 8)	GGTTAGGTTGTAGTATTG (SEQ ID NO: 346)
73	GPIb beta (SEQ ID NO: 8)	ATGGGTTTCGGTGAGTTT (SEQ ID NO: 599)
74	ID NO: 8)	ATGGGTTTTGGTGAGTTT (SEQ ID NO: 600)
75	NO: 9)	ATAGTAGTCGGGTGTTGG (SEQ ID NO: 347)
76	NO: 9)	ATAGTAGTTGGGTGTTGG (SEQ ID NO: 348)
77	NO: 9)	GTGTTAGTCGTTTAGGGT (SEQ ID NO: 597)
78	NO: 9)	GTGTTAGTTTTAGGGT (SEQ ID NO: 598)
79	NO: 9)	TAGTTGTTCGTTTGGGTT (SEQ ID NO: 349)
80	NO: 9)	TAGTTGTTTGGGTT (SEQ ID NO: 350)
81	NO: 9)	GGTTATTACGGATAAATA (SEQ ID NO: 351)
82	NO: 9)	GGTTATTATGGATAAATA (SEQ ID NO: 352)
83	CDH3 (SEQ ID NO: 10)	AAATTAGTCGGGTGTGGT (SEQ ID NO: 353)
84	CDH3 (SEQ ID NO: 10)	AAATTAGTTGGGTGTGGT (SEQ ID NO: 354)
85	CDH3 (SEQ ID NO: 10)	TGTGGTGGCGTAAGTTTG (SEQ ID NO: 355)
86	CDH3 (SEQ ID NO: 10)	TGTGGTGGTGTAAGTTTG (SEQ ID NO: 356)
87	CDH3 (SEQ ID NO: 10)	TTTAGAAACGCGGTTTTT (SEQ ID NO: 357)
88	CDH3 (SEQ ID NO: 10)	TTTAGAAATGCGGTTTTT (SEQ ID NO: 358)
89	CDH3 (SEQ ID NO: 10)	GGAGTTTTCGTTTTTAGT (SEQ ID NO: 359)
90	CDH3 (SEQ ID NO: 10)	GGAGTTTTTGTTTTTAGT (SEQ ID NO: 360)
91	CDH3 (SEQ ID NO: 10)	TAGAATTGCGAGATAGAG (SEQ ID NO: 361)
92	CDH3 (SEQ ID NO: 10)	TAGAATTGTGAGATAGAG (SEQ ID NO: 362)
93		TTGAGGGTCGTTAGGTGG (SEQ ID NO: 363)
94		TTGAGGGTTGTTAGGTGG (SEQ ID NO: 364)

	NO: 11)	
95	MYCL1 (SEQ ID	TTTTAGTTCGGAGTGGGT (SEQ ID NO: 365)
	NO: 11)	
96		TTTTAGTTTGGAGTGGGT (SEQ ID NO: 366)
0.77	NO: 11)	L COMMENT COMM
97		AGTTTAGTCGGTTGGTAT (SEQ ID NO: 367)
00	NO: 11)	A CTTT A CTTCCTTCCT AT (CTC ID NO. 260)
98	NO: 11)	AGTTTAGTTGGTTAT (SEQ ID NO: 368)
99		GGGGTTATCGGGGATTGA (SEQ ID NO: 369)
	NO: 11)	COCCITITION (BEQ ID NO. 307)
100		GGGGTTATTGGGGATTGA (SEQ ID NO: 370)
	NO: 11)	
101	ELK1 (SEQ ID	TTTGTTTTCGTTGAGTAG (SEQ ID NO: 371)
	NO: 12)	
102	ELK1 (SEQ ID	TTTGTTTGTTGAGTAG (SEQ ID NO: 372)
100	NO: 12)	
103	ELK1 (SEQ ID	TTTATTTTCGTTTTTGGG (SEQ ID NO: 373)
104	NO: 12) ELK1 (SEQ ID	TTTATTTTGTTTTTGGG (SEQ ID NO: 374)
104	NO: 12)	TIATITITITITITITIOG (SEQ ID NO. 374)
105	ELK1 (SEQ ID	GAAGGGTTCGTTTTTAA (SEQ ID NO: 375)
	NO: 12)	
106	ELK1 (SEQ ID	GAAGGGTTTGTTTTTAA (SEQ ID NO: 376)
	NO: 12)	
107	ELK1 (SEQ ID	ATTAATAGCGTTTTGGTT (SEQ ID NO: 377)
	NO: 12)	
108	ELK1 (SEQ ID	ATTAATAGCGTTTTGGTT (SEQ ID NO: 378)
1.00	NO: 12)	
109	ABL1 (SEQ ID NO: 13)	GTTTTTTCGGGTTTTTT (SEQ ID NO: 379)
110	ABL1 (SEQ ID	GTTTTTTTGGGTTTTTT (SEQ ID NO: 380)
110	NO: 13)	G11111111000111111 (SEQ 1D NO. 580)
111	APC (SEQ ID	TATTAGAGCGTTTTAAAG (SEQ ID NO: 381)
	NO: 14)	(
112	APC (SEQ ID	TATTAGAGTGTTTTAAAG (SEQ ID NO: 382)
	NO: 14)	
113	APC (SEQ ID	GTTTTTTCGATTTGGGT (SEQ ID NO: 383)
114	NO: 14)	
114	APC (SEQ ID	GTTTTTTTGATTTGGGT (SEQ ID NO: 384)
115	NO: 14) BCL2 (SEQ ID	AGTGTTTCGCGTGATTGA (SEQ ID NO: 385)
110	NO: 15)	AGIGITICOCOTOATIOA (SEQ ID NO: 383)
116		AGTGTTTTGTGTGATTGA (SEQ ID NO: 386)
	NO: 15)	in the state of th
117		AGTTGGGGCGAGAGGTGT (SEQ ID NO: 567)
	NO: 15)	
118		AGTTGGGGTGAGAGGTGT (SEQ ID NO: 568)
	NO: 15)	
119		TAAGTTGTCGTAGAGGGG (SEQ ID NO: 387)
	NO: 15)	

120	BCL2 (SEQ ID NO: 15)	TAAGTTGTTGTAGAGGGG (SEQ ID NO: 388)
121	BCL2 (SEQ ID NO: 15)	AGGGGTTACGAGTGGGAT (SEQ ID NO: 389)
122	BCL2 (SEQ ID NO: 15)	AGGGGTTATGAGTGGGAT (SEQ ID NO: 390)
123	BCL2 (SEQ ID NO: 15)	AGGATTTCGTCGTTGTAG (SEQ ID NO: 391)
124	BCL2 (SEQ ID NO: 15)	AGGATTTTGTTGTAG (SEQ ID NO: 392)
125	CDH1 (SEQ ID NO: 16)	AGGGGTGCGTGGTTGTA (SEQ ID NO: 393)
126	CDH1 (SEQ ID NO: 16)	AGGGGGTGCATGGTTGTA (SEQ ID NO: 394)
127	CDH1 (SEQ ID NO: 16)	AGTTTCGACGTTATTGAG (SEQ ID NO: 395)
128	CDH1 (SEQ ID NO: 16)	AGTTTCAACATTATTGAG (SEQ ID NO: 396)
129	CDH1 (SEQ ID NO: 16)	AGAGGTTGCGGTTTTAAG (SEQ ID NO: 397)
130	CDH1 (SEQ ID NO: 16)	AGAGGTTGCAGTTTTAAG (SEQ ID NO: 398)
131	CDH1 (SEQ ID NO: 16)	AGGGGATTCGGGGTATTT (SEQ ID NO: 399)
132	CDH1 (SEQ ID NO: 16)	AGGGGATTCAGGGTATTT (SEQ ID NO: 400)
133	CDKN1A (SEQ ID NO: 17)	TGGGTTAGCGGTGAGTTA (SEQ ID NO: 401)
134	CDKN1A (SEQ ID NO: 17)	TGGGTTAGTGGTGAGTTA (SEQ ID NO: 402)
135	CDKN1A (SEQ ID NO: 17)	TTGTAGTACGCGAGGTTT (SEQ ID NO: 403)
136	CDKN1A (SEQ ID NO: 17)	TTGTAGTATGTGAGGTTT (SEQ ID NO: 404)
137	CDKN1A (SEQ ID NO: 17)	TTGGAATTCGGTTAGGTT (SEQ ID NO: 405)
138	CDKN1A (SEQ ID NO: 17)	TTGGAATTTGGTTAGGTT (SEQ ID NO: 406)
139	CDKN1A (SEQ ID NO: 17)	AGTTGGTTCGGCGTTGGG (SEQ ID NO: 589)
140	CDKN1A (SEQ ID NO: 17)	AGTTGGTTTGGG (SEQ ID NO: 590)
141	CDKN1B (SEQ ID NO: 18)	AAGAGAAACGTTGGAATA (SEQ ID NO: 407)
142	CDKN1B (SEQ ID NO: 18)	AAGAGAAATGTTGGAATA (SEQ ID NO: 408)
143	CDKN1B (SEQ ID NO: 18)	TTTGATTTCGAGGGGAGT (SEQ ID NO: 409)
144	CDKN1B (SEQ ID NO: 18)	TTTGATTTTGAGGGGAGT (SEQ ID NO: 410)
145	CDKN1B (SEQ	GTATTTGGCGGTTGGATT (SEQ ID NO: 411)

	ID NO: 18)	
146	CDKN1B (SEQ	GTATTTGGTGGTTGGATT (SEQ ID NO: 412)
	ID NO: 18)	
147	CDKN1B (SEQ	TATAATTTCGGGAAAGAA (SEQ ID NO: 413)
1.40	ID NO: 18)	
148	CDKN1B (SEQ	TATAATTTTGGGAAAGAA (SEQ ID NO: 414)
140	ID NO: 18)	CCA CTTTTCCCTTCATTC (GEO ID NO. 415)
149	1	GGAGTTTTCGGTTGATTG (SEQ ID NO: 415)
150	NO: 19)	GGAGTTTTTGGTTGATTG (SEQ ID NO: 416)
130	NO: 19)	OUAUTTTTUUTTUATTU (SEQ ID NO. 410)
151		TTGTTTAACGTATCGAAT (SEQ ID NO: 417)
	NO: 19)	
152		TTGTTTAATGTATTGAAT (SEQ ID NO: 418)
	NO: 19)	
153	CDKN2a (SEQ ID	AATAGTTACGGTCGGAGG (SEQ ID NO: 419)
	NO: 19)	
154		AATAGTTATGGTTGGAGG (SEQ ID NO: 420)
	NO: 19)	
155	, , ,	ATATTTAGCGAGTAGTGT (SEQ ID NO: 421)
150	ID NO: 20)	A TA TTT A CTC A CTA CTCT (CTC ATD NO. 400)
156	, ,	ATATTTAGTGAGTAGTGT (SEQ ID NO: 422)
157	ID NO: 20) CDKN2B (SEQ	TGGGGAGACGTCGGTTTT (SEQ ID NO: 423)
137	ID NO: 20)	SEQ ID NO. 423)
158	CDKN2B (SEQ	TGGGGAGATGTTGGTTTT (SEQ ID NO: 424)
, , ,	ID NO: 20)	
159	CDKN2B (SEQ	TTATTGTACGGGGTTTTA (SEQ ID NO: 425)
	ID NO: 20)	
160	CDKN2B (SEQ	TTATTGTATGGGGTTTTA (SEQ ID NO: 426)
	ID NO: 20)	
161	, ,	TAGAAGGACGACGGAGG (SEQ ID NO: 577)
1.60	ID NO: 20)	
162		TAGAAGGATGATGGGAGG (SEQ ID NO: 578)
163	ID NO: 20)	A C A C A CTCCCCTCCC A CT A (GEO ID NO. 575)
103	CDKN2B (SEQ ID NO: 20)	AGAGAGTGCGTCGGAGTA (SEQ ID NO: 575)
164	··· · · · · · · · · · · · · · · · · ·	AGAGAGTGTGTTGGAGTA (SEQ ID NO: 576)
104	ID NO: 20)	AGAGAGIGIGIIGGAGIA (BEQ ID NO. 570)
165		AATGTTTTCGTACGTAGG (SEQ ID NO: 427)
	NO: 21)	(524 15 1/6. 127)
166		AATGTTTTGTATGTAGG (SEQ ID NO: 428)
	NO: 21)	· · · ·
167	FOS (SEQ ID	TATATGGTCGAGAAAAAT (SEQ ID NO: 429)
1.55	NO: 21)	
168	FOS (SEQ ID	TATATGGTTGAGAAAAAT (SEQ ID NO: 430)
1.60	NO: 21)	TTTL CT LTCCT L L CT LC (CT C TT CT C TC CT C TT CT C TT CT CT C TT CT C
169		TTTAGTATCGTAAAGTAG (SEQ ID NO: 431)
170	NO: 21) FOS (SEQ ID	TTTAGTATTGTAAAGTAG (SEQ ID NO: 432)
170	NO: 21)	I I IAGIAI I GIAAAGIAG (SEQ ID NO: 432)
	μ.(0, Δ1)	

171	FOS (SEQ ID NO: 21)	GTATTGTTCGAGTTCGAG (SEQ ID NO: 433)
172	FOS (SEQ ID NO: 21)	GTATTGTTTGAGTTTGAG (SEQ ID NO: 434)
173	GSTP1 (SEQ ID NO: 22)	GGTTTTTCGGTTAGTTG (SEQ ID NO: 435)
174	GSTP1 (SEQ ID NO: 22)	GGTTTTTTGGTTAGTTG (SEQ ID NO: 436)
175	GSTP1 (SEQ ID NO: 22)	TTTTAGGGCGTTTTTTTG (SEQ ID NO: 437)
176	GSTP1 (SEQ ID NO: 22)	TTTTAGGGTGTTTTTTTG (SEQ ID NO: 438)
177	GSTP1 (SEQ ID NO: 22)	GTAGTTTCGTTATTAGT (SEQ ID NO: 439)
178	GSTP1 (SEQ ID NO: 22)	GTAGTTTTTGTTATTAGT (SEQ ID NO: 440)
179	HIC-1 (SEQ ID NO: 23)	ATGATTCGTCGTGGGTTT (SEQ ID NO: 441)
180	HIC-1 (SEQ ID NO: 23)	ATGATTTGTTGTGGGTTT (SEQ ID NO: 442)
181	HIC-1 (SEQ ID NO: 23)	AGGAGATTCGAAAGTTTA (SEQ ID NO: 443)
182 183	HIC-1 (SEQ ID NO: 23) HIC-1 (SEQ ID	AGGAGATTTGAAAGTTTA (SEQ ID NO: 444) GGGTTTTACGTGGTTGTT (SEQ ID NO: 445)
184	NO: 23) HIC-1 (SEQ ID	GGGTTTTACGTGGTTGTT (SEQ ID NO: 445)
185	NO: 23) HIC-1 (SEQ ID	TTTTAGAGCGTTAGGGTT (SEQ ID NO: 447)
186	NO: 23) HIC-1 (SEQ ID	TTTTAGAGCGTTAGGGTT (SEQ ID NO: 447) TTTTAGAGTGTTAGGGTT (SEQ ID NO: 448)
187	NO: 23) MGMT (SEQ ID	TAAGGATACGAGTTATAT (SEQ ID NO: 449)
188	NO: 24) MGMT (SEQ ID	TAAGGATATGAGTTATAT (SEQ ID NO: 450)
189	NO: 24) MGMT (SEQ ID	TTGGAGAGCGGTTGAGTT (SEQ ID NO: 451)
190	NO: 24) MGMT (SEQ ID	TTGGAGAGTGGTTGAGTT (SEQ ID NO: 452)
191	NO: 24) MGMT (SEQ ID	TAGGTTATCGGTGATTGT (SEQ ID NO: 453)
192	NO: 24) MGMT (SEQ ID	TAGGTTATTGGTGATTGT (SEQ ID NO: 454)
193	NO: 24) MGMT (SEQ ID	AGTAGGATCGGGATTTTT (SEQ ID NO: 455)
194	NO: 24) MGMT (SEQ ID	AGTAGGATTGGGATTTTT (SEQ ID NO: 456)
195	NO: 24) MLH1 (SEQ ID	TTGAGAAGCGTTAAGTAT (SEQ ID NO: 457)
196	NO: 25) MLH1 (SEQ ID	TTGAGAAGTGTTAAGTAT (SEQ ID NO: 458)
170	TATELLE (SEG ID	ITTOAUAATUTTAAUTTI (DEQ ID NO. 430)

]	NO: 25)	
197	MLH1 (SEQ ID NO: 25)	TTAGGTAGCGGGTAGTAG (SEQ ID NO: 573)
198	MLH1 (SEQ ID NO: 25)	TTAGGTAGTGGGTAGTAG (SEQ ID NO: 574)
199	MLH1 (SEQ ID NO: 25)	GTAGTAGTCGTTTTAGGG (SEQ ID NO: 559)
200	MLH1 (SEQ ID NO: 25)	GTAGTAGTTGTTTTAGGG (SEQ ID NO: 560)
201	MLH1 (SEQ ID NO: 25)	ATAGTTGTCGTTGAAGGG (SEQ ID NO: 459)
202	MLH1 (SEQ ID NO: 25)	ATAGTTGTTGAAGGG (SEQ ID NO: 460)
203	MLH1 (SEQ ID NO: 25)	GGGTTATTCGGCGGTTGG (SEQ ID NO: 461)
204	MLH1 (SEQ ID NO: 25)	GGGTTATTTGGTGGTTGG (SEQ ID NO: 462)
205	MOS (SEQ ID NO: 26)	AGTAGTTTCGTAGGTAGT (SEQ ID NO: 463)
206	MOS (SEQ ID NO: 26)	AGTAGTTTCATAGGTAGT (SEQ ID NO: 464)
207	MOS (SEQ ID NO: 26)	GTAAGTCGTTTTGTATAT (SEQ ID NO: 465)
208	MOS (SEQ ID NO: 26)	GTAAGTCATTTTGTATAT (SEQ ID NO: 466)
209	MOS (SEQ ID NO: 26)	AGGAATATCGCGGTAAGT (SEQ ID NO: 467)
210	MOS (SEQ ID NO: 26)	AGGAATATCACAGTAAGT (SEQ ID NO: 468)
211	MOS (SEQ ID NO: 26)	ATGTTAGTCGGTTTTTGG (SEQ ID NO: 469)
212	MOS (SEQ ID NO: 26)	ATGTTAGTCAGTTTTTGG (SEQ ID NO: 470)
213	MYC (SEQ ID NO: 27)	TTAGAGTGTTCGGTTGTT (SEQ ID NO: 585)
214	MYC (SEQ ID NO: 27)	TTAGAGTGTTTGGTTGTT (SEQ ID NO: 586)
215	MYC (SEQ ID NO: 27)	AGGATTTTCGAGTTGTGT (SEQ ID NO: 471)
216	MYC (SEQ ID NO: 27)	AGGATTTTTGAGTTGTGT (SEQ ID NO: 472)
217	MYC (SEQ ID NO: 27)	GAGGGATCGCGTTGAGTA (SEQ ID NO: 473)
218	MYC (SEQ ID NO: 27)	GAGGGATTGTGTTGAGTA (SEQ ID NO: 474)
219	MYC (SEQ ID NO: 27)	AATTTTAGCGAGAGGTAG (SEQ ID NO: 475)
220	MYC (SEQ ID NO: 27)	AATTTTAGTGAGAGGTAG (SEQ ID NO: 476)
221	MYC (SEQ ID NO: 27)	TTGTGGGCGTTTTGGGAA (SEQ ID NO: 477)

222	MYC (SEQ ID NO: 27)	TTGTGGGTGTTTTGGGAA (SEQ ID NO: 478)
223	PTEN (SEQ ID NO: 28)	GGATTTTGCGTTCGTATT (SEQ ID NO: 479)
224	PTEN (SEQ ID NO: 28)	GGATTTTGTGTTTGTATT (SEQ ID NO: 480)
225	PTEN (SEQ ID NO: 28)	AGAGTTATCGTTTTGTTT (SEQ ID NO: 481)
226	PTEN (SEQ ID NO: 28)	AGAGTTATTGTTTT (SEQ ID NO: 482)
227	PTEN (SEQ ID NO: 28)	TGATGTGGCGGGATTTTT (SEQ ID NO: 483)
228	PTEN (SEQ ID NO: 28)	TGATGTGGTGGGATTTTT (SEQ ID NO: 484)
229	PTEN (SEQ ID NO: 28)	TTTTTATGCGTTGCGGTA (SEQ ID NO: 485)
230	PTEN (SEQ ID NO: 28)	TTTTTATGTGTTGTGGTA (SEQ ID NO: 486)
231	NO: 29)	ATTAGTGTCGTTGTTAAG (SEQ ID NO: 487)
232	NO: 29)	ATTAGTGTTGTTAAG (SEQ ID NO: 488)
233	NO: 29)	AGATTATACGGATAAGGG (SEQ ID NO: 489)
234	NO: 29)	AGATTATATGGATAAGGG (SEQ ID NO: 490)
235	NO: 30)	ATTTGGAGTGAGGAATTT (SEQ ID NO: 491)
236	NO: 30)	ATTTGGAGTGAGGAATTT (SEQ ID NO: 492)
237	NO: 30)	TTGAAAGTCGGTTAAAGT (SEQ ID NO: 493)
238 239	NO: 30)	TTGAAAGTTGGTTAAAGT (SEQ ID NO: 494)
	NO: 30)	AAAGTTTTCGGAGGGGTT (SEQ ID NO: 495)
240 241	NO: 30)	AAAGTTTTTGGAGGGGTT (SEQ ID NO: 496) GGTAGTTACGAGAGAGTT (SEQ ID NO: 497)
242	NO: 30)	GGTAGTTACGAGAGAGTT (SEQ ID NO: 497) GGTAGTTATGAGAGAGTT (SEQ ID NO: 498)
	NO: 30)	, , ,
243	NO: 30)	GTTGGACGTCGAGGAGAG (SEQ ID NO: 499)
244	NO: 30)	GTTGGATGTTGAGGAGAG (SEQ ID NO: 500)
245	NO: 31)	TTTGGTGCGCGTAGAGAA (SEQ ID NO: 581)
246	NO: 31)	TTTGGTGTGTAGAGAA (SEQ ID NO: 582)
247	TP73 (SEQ ID	GAGTGTTCGCGTTTTGGG (SEQ ID NO: 593)

	NO: 31)	·
248	TP73 (SEQ ID	GAGTGTTTGTGTTTTGGG (SEQ ID NO: 594)
249	NO: 31) TP73 (SEQ ID	AAGTTACGGGTTTTATTG (SEQ ID NO: 587)
279	NO: 31)	AAGTTACGGGTTTTATTG (SEQ ID NO. 367)
250	TP73 (SEQ ID NO: 31)	AAGTTATGGGTTTTATTG (SEQ ID NO: 588)
251	TP73 (SEQ ID NO: 31)	TAAGTAGCGTCGTTATTG (SEQ ID NO: 501)
252	TP73 (SEQ ID NO: 31)	TAAGTAGTGTTGTTATTG (SEQ ID NO: 502)
253	TP73 (SEQ ID NO: 31)	GGAAGTTTCGATGGTTTA (SEQ ID NO: 503)
254	TP73 (SEQ ID NO: 31)	GGAAGTTTTGATGGTTTA (SEQ ID NO: 504)
255	CDKN1C (SEQ ID NO: 32)	ATGAAGAACGGTTAAGGG (SEQ ID NO: 591)
256	CDKN1C (SEQ ID NO: 32)	ATGAAGAATGGTTAAGGG (SEQ ID NO: 592)
257	CDKN1C (SEQ ID NO: 32)	TTAAGTTACGGTTATTAG (SEQ ID NO: 505)
258	CDKN1C (SEQ ID NO: 32)	TTAAGTTATGGTTATTAG (SEQ ID NO: 506)
259	CDKN1C (SEQ ID NO: 32)	TTAGTGTTCGTTTGGAAT (SEQ ID NO: 507)
260	CDKN1C (SEQ ID NO: 32)	TTAGTGTTTGGAAT (SEQ ID NO: 508)
261	GSK3ß (SEQ ID NO: 33)	GGGTAAAGCGCGGATATT (SEQ ID NO: 509)
262	GSK3ß (SEQ ID NO: 33)	GGGTAAAGTGTGGATATT (SEQ ID NO: 510)
263	GSK3ß (SEQ ID NO: 33)	TATGTTTCGGCGAATGG (SEQ ID NO: 511)
264	GSK3ß (SEQ ID NO: 33)	TATGTTTTTGGTGAATGG (SEQ ID NO: 512)
265	GSK3ß (SEQ ID NO: 33)	GGGGAATAGTCGAGGAGT (SEQ ID NO: 513)
266	GSK3ß (SEQ ID NO: 33)	GGGGAATAGTTGAGGAGT (SEQ ID NO: 514)
267	GSK3ß (SEQ ID NO: 33)	AGGAGTCGTTGTTTGGGG (SEQ ID NO: 515)
268	GSK3ß (SEQ ID NO: 33)	AGGAGTTGTTTGGGG (SEQ ID NO: 516)
269	ESR1 (SEQ ID NO: 34)	AGATATATCGGAGTTTGG (SEQ ID NO: 517)
270	ESR1 (SEQ ID NO: 34)	AGATATATTGGAGTTTGG (SEQ ID NO: 518)
271	ESR1 (SEQ ID NO: 34)	GTTTGGTACGGGGTATAT (SEQ ID NO: 519)
272	ESR1 (SEQ ID NO: 34)	GTTTGGTATGGGGTATAT (SEQ ID NO: 520)

273	ESR1 (SEQ ID NO: 34)	TTAGTAGCGACGATAAGT (SEQ ID NO: 521)
274	ESR1 (SEQ ID NO: 34)	TTAGTAGTGATGATAAGT (SEQ ID NO: 522)
275	ESR1 (SEQ ID NO: 34)	TATGAGTTCGGGAGATTA (SEQ ID NO: 523)
276	ESR1 (SEQ ID NO: 34)	TATGAGTTTGGGAGATTA (SEQ ID NO: 524)
277	ESR1 (SEQ ID NO: 34)	TGGAGGTTCGGGAGTTTA (SEQ ID NO: 525)
278	ESR1 (SEQ ID NO: 34)	TGGAGGTTTGGGAGTTTA (SEQ ID NO: 526)
279	APAF1 (SEQ ID NO: 35)	TTTGGTATCGTTTAGAGT (SEQ ID NO: 527)
280	APAF1 (SEQ ID NO: 35)	TTTGGTATTGTTTAGAGT (SEQ ID NO: 528)
281	APAF1 (SEQ ID NO: 35)	GTATGAGTCGTGGTAGGA (SEQ ID NO: 529)
282	APAF1 (SEQ ID NO: 35)	GTATGAGTTGTGGTAGGA (SEQ ID NO: 530)
283	APAF1 (SEQ ID NO: 35)	GTGGATTCGGCGGGATTT (SEQ ID NO: 531)
284	APAF1 (SEQ ID NO: 35)	GTGGATTTGGTGGGATTT (SEQ ID NO: 532)
285	APAF1 (SEQ ID NO: 35)	TTTAGAGGCGGAGAAGAA (SEQ ID NO: 533)
286	APAF1 (SEQ ID NO: 35)	TTTAGAGGTGGAGAAGAA (SEQ ID NO: 534)
287	APAF1 (SEQ ID NO: 35)	GAAGAGGTAGCGAGTGGA (SEQ ID NO: 535)
288	APAF1 (SEQ ID NO: 35)	GAAGAGGTAGTGAGTGGA (SEQ ID NO: 536)
289·	BAK1 (SEQ ID NO: 36)	TAGGTTGTCGGTTTGTGC (SEQ ID NO: 537)
290	BAK1 (SEQ ID NO: 36)	TAGGTTGTTGGTGC (SEQ ID NO: 538)
291	BAK1 (SEQ ID NO: 36)	TTTGTATTCGGTGGTTAT (SEQ ID NO: 539)
292	BAK1 (SEQ ID NO: 36)	TTTGTATTTGGTGGTTAT (SEQ ID NO: 540)
293	BAK1 (SEQ ID NO: 36)	GGAGTTTCGCGGGTTTTT (SEQ ID NO: 565)
294	BAK1 (SEQ ID NO: 36)	GGAGTTTTGTGGGTTTTT (SEQ ID NO: 566)
295	BAK1 (SEQ ID NO: 36)	TAGGATTTCGGTAGGTAA (SEQ ID NO: 541)
296	BAK1 (SEQ ID NO: 36)	TAGGATTTTGGTAGGTAA (SEQ ID NO: 542)
297	BAX (SEQ ID NO: 37)	AGTTTGGGCGTGGGTTAT (SEQ ID NO: 543)
298	BAX (SEQ ID	AGTTTGGGTGTGGGTTAT (SEQ ID NO: 544)

	NO: 37)	
299	BAX (SEQ ID NO: 37)	ATTAGAGTTGCGATTGGA (SEQ ID NO: 545)
300	BAX (SEQ ID NO: 37)	ATTAGAGTTGTGATTGGA (SEQ ID NO: 546)
301	BAX (SEQ ID NO: 37)	GTATTTATCGGGAGATGT (SEQ ID NO: 547)
302	BAX (SEQ ID NO: 37)	GTATTTATTGGGAGATGT (SEQ ID NO: 548)
303	BAX (SEQ ID NO: 37)	TTTAGAGGCGGGGGTGAG (SEQ ID NO: 549)
304	BAX (SEQ ID NO: 37)	TTTAGAGGTGGGGTGAG (SEQ ID NO: 550)
305	HOXA5 (SEQ ID NO: 38)	AGTTAGTCGGGTTTTAAG (SEQ ID NO: 551)
306	HOXA5 (SEQ ID NO: 38)	AGTTAGTCAGGTTTTAAG (SEQ ID NO: 552)
307	HOXA5 (SEQ ID NO: 38)	TTATAGGGTTCGGTTTTT (SEQ ID NO: 553)
308	HOXA5 (SEQ ID NO: 38)	TTATAGGGTTCAGTTTTT (SEQ ID NO: 554)
309	HOXA5 (SEQ ID NO: 38)	TTTTAAGGCGAGGTTAAA (SEQ ID NO: 555)
310	HOXA5 (SEQ ID NO: 38)	TTTTAAGGCAAGGTTAAA (SEQ ID NO: 556)
311	HOXA5 (SEQ ID NO: 38)	ATGATAGGCGTTTATTAA (SEQ ID NO: 557)
312	HOXA5 (SEQ ID NO: 38)	ATGATAGGCATTTATTAA (SEQ ID NO: 558)

<u>Table 3: Oligonucleotides used in differentiation of MCL, DLBCL & CLL/SLL from FL I and II.</u>

No:	Gene	Oligo:
2157:1808A	MLH1	GTAGTAGTCGTTTTAGGG
	(SEQ ID NO: 25)	(SEQ ID NO: 559)
1012:2491A18	ELK1	GAAGGGTTCGTTTTTAA
8	(SEQ ID NO: 12)	(SEQ ID NO: 375)
1012:2491B188	ELK1	GAAGGGTTTGTTTTTAA
	(SEQ ID NO: 12)	(SEQ ID NO: 376)
2428:2120A18	ESR	TGGAGGTTCGGGAGTTTA
8	(SEQ ID NO: 34)	(SEQ ID NO: 525)
2428:2120B188	ESR	TGGAGGTTTGGGAGTTTA
,	(SEQ ID NO: 34)	(SEQ ID NO: 526)
2157:1808B	MLH1	GTAGTAGTTGTTTTAGGG
	(SEQ ID NO: 25)	(SEQ ID NO: 560)
130:175A	GPIb beta	GTGGGAGCGGAAGTTTGA
	(SEQ ID NO: 8)	(SEQ ID NO: 561)
130:175B	GPIb beta	GTGGGAGTGGAAGTTTGA

No:	Gene	Oligo:
	(SEQ ID NO: 8)	(SEQ ID NO: 562)
88:276A	CDK4	GGAAGGGTCGTTTAAGGG
	(SEQ ID NO: 5)	(SEQ ID NO: 563)
88:276B	CDK4	GGAAGGGTTGTTTAAGGG
	(SEQ ID NO: 5)	(SEQ ID NO: 564)
2494:1989A	BAK1	GGAGTTTCGCGGGTTTTT
	(SEQ ID NO: 36)	(SEQ ID NO: 565)
2494:1989B	BAK1	GGAGTTTTGTGGGTTTTT
	(SEQ ID NO: 36)	(SEQ ID NO: 566)
2013:1990A	BCL2	AGTTGGGGCGAGAGGTGT
	(SEQ ID NO: 15)	(SEQ ID NO: 567)
2013:1990B	BCL2	AGTTGGGGTGAGAGGTGT
	(SEQ ID NO: 15)	(SEQ ID NO: 568)
87:168A	AR	AGAGGTTGCGTTTTAGAG
	(SEQ ID NO: 4)	(SEQ ID NO: 569)
87:168B	AR	AGAGGTTGTGTTTTAGAG
	(SEQ ID NO: 4)	(SEQ ID NO: 570)
89:451A	MOS	TATGGAGTTCGGTGGTAA
	(SEQ ID NO: 26)	(SEQ ID NO: 571)
89:451B	MOS	TATGGAGTTTGGTGGTAA
	(SEQ ID NO: 26)	(SEQ ID NO: 572)
2157:1797A	MLH1	TTAGGTAGCGGGTAGTAG
	(SEQ ID NO: 25)	(SEQ ID NO: 573)
2157:1797B	MLH1	TTAGGTAGTGGGTAGTAG
	(SEQ ID NO: 25)	(SEQ ID NO: 574)
2036:2234A	CDKN2B	AGAGAGTGCGTCGGAGTA
	(SEQ ID NO: 20)	(SEQ ID NO: 575)
2036:2234B	CDKN2B	AGAGAGTGTTGGAGTA
	(SEQ ID NO: 20)	(SEQ ID NO: 576)
2036:2183A	CDKN2B	TAGAAGGACGACGGGAGG
	(SEQ ID NO: 20)	(SEQ ID NO: 577)
2036:2183B	CDKN2B	TAGAAGGATGATGGGAGG
	(SEQ ID NO: 20)	(SEQ ID NO: 578)

Table 4: Oligonucleotides used in differentiation of MCL, DLBCL & CLL/SLL from FL I and II.

No:	Gene	Oligo:
2157:1808A	MLH1	GTAGTAGTCGTTTTAGGG
	(SEQ ID NO: 25)	(SEQ ID NO: 559)
2428:2120A18	ESR	TGGAGGTTCGGGAGTTTA
8		(SEQ ID NO: 525)

No:	Gene	Oligo:
	(SEQ ID NO: 34)	
2428:2120B188	ESR	TGGAGGTTTGGGAGTTTA
	(SEQ ID NO: 34)	(SEQ ID NO: 526)
1010:753A188	MYC1	AGTTTAGTCGGTTGGTAT
	(SEQ ID NO: 11)	(SEQ ID NO: 367)
1010:753B188	MYC1	AGTTTAGTTGGTTAT
	(SEQ ID NO: 11)	(SEQ ID NO: 368)
2013:1990A18	BCL2	AGTTGGGGCGAGAGGTGT
8	(SEQ ID NO: 15)	(SEQ ID NO: 567)
2013:1990B188	BCL2	AGTTGGGGTGAGAGGTGT
	(SEQ ID NO: 15)	(SEQ ID NO: 568)
2157:1808A18	MLH1	GTAGTAGTCGTTTTAGGG
8	(SEQ ID NO: 25)	(SEQ ID NO: 559)
2157:1808B188	MLH1	GTAGTAGTTGTTTTAGGG
	(SEQ ID NO: 25)	(SEQ ID NO: 560)
130:269A188	GPIb beta	GGTTAGGTCGTAGTATTG
	(SEQ ID NO: 8)	(SEQ ID NO: 345)
130:269B188	GPIb beta	GGTTAGGTTGTAGTATTG
	(SEQ ID NO: 8)	(SEQ ID NO: 346)
2322:1213A18	TP73	GAGTGTTCGCGTTTTGGG
7	(SEQ ID NO: 31)	(SEQ ID NO: 593)

No:	Gene	Oligo:
2322:1213B187	TP73	GAGTGTTTGTGTTTTGGG
	(SEQ ID NO: 31)	(SEQ ID NO: 594)
	(SEQIDIO. 31)	
2157:1808B	MLH1	GTAGTAGTTGTTTTAGGG
	(SEQ ID NO: 25)	(SEQ ID NO: 560)
130:175A	GPIb beta	GTGGGAGCGGAAGTTTGA
	(SEQ ID NO: 8)	(SEQ ID NO: 561)
130:175B	GPIb beta	GTGGGAGTGGAAGTTTGA
	(SEQ ID NO: 8)	(SEQ ID NO: 562)
\		
88:276A	CDK4	GGAAGGGTCGTTTAAGGG
	(SEQ ID NO: 5)	(SEQ ID NO: 563)
88:276B	CDK4	GGAAGGGTTGTTTAAGGG
	(SEQ ID NO: 5)	(SEQ ID NO: 564)
2494:1989A	BAK1 ·	GGAGTTTCGCGGGTTTTT
	(SEQ ID NO: 36)	(SEQ ID NO: 565)
2404 40007	D 1774	
2494:1989B	BAK1	GGAGTTTTGTGGGTTTTT
	(SEQ ID NO: 36)	(SEQ ID NO: 566)
2013:1990A	BCL2	AGTTGGGGCGAGAGGTGT
	(SEQ ID NO: 15)	(SEQ ID NO: 567)
2013:1990B	BCL2	AGTTGGGGTGAGAGGTGT
2013.179013		(SEQ ID NO: 568)
·	(SEQ ID NO: 15)	(OEQ ID NO. 300)
87:168A	AR	AGAGGTTGCGTTTTAGAG
	(SEQ ID NO: 4)	(SEQ ID NO: 569)
87:168B	AR	AGAGGTTGTGTTTTAGAG
,		

No:	Gene	Oligo:
	(SEQ ID NO: 4)	(SEQ ID NO: 570)
89:451A	MOS	TATGGAGTTCGGTGGTAA
	(SEQ ID NO: 26)	(SEQ ID NO: 571)
89:451B	MOS	TATGGAGTTTGGTGGTAA
	(SEQ ID NO: 26)	(SEQ ID NO: 572)
2157:1797A	MLH1	TTAGGTAGCGGGTAGTAG
	(SEQ ID NO: 25)	(SEQ ID NO: 573)
2157:1797B	MLH1	TTAGGTAGTGGGTAGTAG
	(SEQ ID NO: 25)	(SEQ ID NO: 574)
2036:2234A	CDKN2B	AGAGAGTGCGTCGGAGTA
	(SEQ ID NO: 20)	(SEQ ID NO: 575)
2036:2234B	CDKN2B	AGAGAGTGTTGGAGTA
	(SEQ ID NO: 20)	(SEQ ID NO: 576)
2036:2183A	CDKN2B	TAGAAGGACGACGGGAGG
	(SEQ ID NO: 20)	(SEQ ID NO: 577)
2036:2183B	CDKN2B	TAGAAGGATGATGGGAGG
	(SEQ ID NO: 20)	(SEQ ID NO: 578)

Table 5: Differentiation of MCL from Follicular Lymphoma

No:	Gene	Oligo:
2322:1082A	TP73	TTTGGTGCGCGTAGAGAA
	(SEQ ID NO: 31)	(SEQ ID NO: 581)
2428:2120A18	ESR	TGGAGGTTCGGGAGTTTA
8	(SEQ ID NO: 34)	(SEQ ID NO: 525)
2428:2120B188	ESR	TGGAGGTTTGGGAGTTTA
		(SEQ ID NO: 526)
1010:753A188	MYC1	AGTTTAGTCGGTTGGTAT
	(SEQ ID NO: 11)	(SEQ ID NO: 367)

No:	Gene	Oligo:
1010:753B188	MYC1	AGTTTAGTTGGTAT
	(SEQ ID NO: 11)	(SEQ ID NO: 368)
1012:2491	ELK1	GAAGGGTTCGTTTTTAA
A188	(SEQ ID NO: 12)	(SEQ ID NO: 375)
1012:2491	ELK1	GAAGGGTTTGTTTTTAA
B188	(SEQ ID NO: 12)	(SEQ ID NO: 376)
2322:906 A188	TP73	TAAGTAGCGTCGTTATTG
	(SEQ ID NO: 31)	(SEQ ID NO: 501)
2322:906 B188	TP73	TAAGTAGTGTTGTTATTG
	(SEQ ID NO: 31)	(SEQ ID NO: 502)
2494:1890	BAK1	TAGGTTGTCGGTTTGTGC
A188	(SEQ ID NO: 36)	(SEQ ID NO: 537)
2494:1890	BAK1	TAGGTTGTTGGTGC
B188	(SEQ ID NO: 36)	(SEQ ID NO: 538)
96:508 A188	RB1	GTGTATTTCGGTTTGGAG
	(SEQ ID NO: 6)	(SEQ ID NO: 601)
96:508 B188	RB1	GTGTATTTTGGTTTGGAG
	(SEQ ID NO: 6)	(SEQ ID NO: 602)
2322:1082B	TP73	TTTGGTGTGTAGAGAA
	(SEQ ID NO: 31)	(SEQ ID NO: 582)
3:1189A	MDR1	TTGGTGGTCGTTTTAAGG
	(SEQ ID NO: 1)	(SEQ ID NO: 583)
3:1189B	MDR1	TTGGTGGTTTTTTAAGG
	(SEQ ID NO: 1)	(SEQ ID NO: 584)
2172:1513A	MYC	TTAGAGTGTTCGGTTGTT
	(SEQ ID NO: 27)	(SEQ ID NO: 585)
2172:1513B	MYC	TTAGAGTGTTTGGTTGTT
	(SEQ ID NO: 27)	(SEQ ID NO: 586)
2322:802A	TP73	AAGTTACGGGTTTTATTG
	(SEQ ID NO: 31)	(SEQ ID NO: 587)
2322:802B	TP73	AAGTTATGGGTTTTATTG
	(SEQ ID NO: 31)	(SEQ ID NO: 588)
2036:2183A	CDKN2B	TAGAAGGACGACGGGAGG
:	(SEQ ID NO: 20)	(SEQ ID NO: 577)
2036:2183B	CDKN2B	TAGAAGGATGATGGGAGG
	(SEQ ID NO: 20)	(SEQ ID NO: 578)
2013:1990A	BCL2	AGTTGGGGCGAGAGGTGT
	(SEQ ID NO: 15)	(SEQ ID NO: 567)
2013:1990B	BCL2	AGTTGGGGTGAGAGGTGT
	(SEQ ID NO: 15)	(SEQ ID NO: 568)
2157:1808A	MLH1	GTAGTAGTCGTTTTAGGG
	(SEQ ID NO: 25)	(SEQ ID NO: 559)
2157:1808B	MLH1	GTAGTAGTTGTTTTAGGG
	(SEQ ID NO: 25)	(SEQ ID NO: 560)
2033:1847A	CDKN1A	AGTTGGTTCGGCGTTGGG
	(SEQ ID NO: 17)	(SEQ ID NO: 589)
2033:1847B	CDKN1A	AGTTGGTTTGGG
	(SEQ ID NO: 17)	(SEQ ID NO: 590)
2350:697A	CDKN1C	ATGAAGAACGGTTAAGGG
	(SEQ ID NO: 32)	(SEQ ID NO: 591)

No:	Gene	Oligo:
2350:697B	CDKN1C	ATGAAGAATGGTTAAGGG
	(SEQ ID NO: 32)	(SEQ ID NO: 592)
130:175A	GPIb beta	GTGGGAGCGGAAGTTTGA
	(SEQ ID NO: 8)	(SEQ ID NO: 561)
130:175B	GPIb beta	GTGGGAGTGGAAGTTTGA
	(SEQ ID NO: 8)	(SEQ ID NO: 562)
87:168A	AR	AGAGGTTGCGTTTTAGAG
	(SEQ ID NO: 4)	(SEQ ID NO: 569)
87:168B	AR	AGAGGTTGTGTTTTAGAG
	(SEQ ID NO: 4)	(SEQ ID NO: 570)
2494:1989A	BAK1	GGAGTTTCGCGGGTTTTT
	(SEQ ID NO: 36)	(SEQ ID NO: 565)
2494:1989B	BAK1	GGAGTTTTGTGGGTTTTT
	(SEQ ID NO: 36)	(SEQ ID NO: 566)
130:165A	GPIb beta	TTTGAGAGCGGGTGGGAG
	(SEQ ID NO: 8)	(SEQ ID NO: 579)
130:165B	GPIb beta	TTTGAGAGTGGGTGGGAG
·	(SEQ ID NO: 8)	(SEQ ID NO: 580)
2036:2234A	CDKN2B	AGAGAGTGCGTCGGAGTA
	(SEQ ID NO: 20)	(SEQ ID NO: 575)
2036:2234B	CDKN2B	AGAGAGTGTGTTGGAGTA
	(SEQ ID NO: 20)	(SEQ ID NO: 576)
2157:1797A	MLH1	TTAGGTAGCGGGTAGTAG
	(SEQ ID NO: 25)	(SEQ ID NO: 573)
2157:1797B	MLH1	TTAGGTAGTGGGTAGTAG
	(SEQ ID NO: 25)	(SEQ ID NO: 574)
2322:1213A	TP73	GAGTGTTCGCGTTTTGGG
	(SEQ ID NO: 31)	(SEQ ID NO: 593)
2322:1213B	TP73	GAGTGTTTGTGTTTTTGGG
	(SEQ ID NO: 31)	(SEQ ID NO: 594)
89:451A	MOS	TATGGAGTTCGGTGGTAA
	(SEQ ID NO: 26)	(SEQ ID NO: 571)
89:451B	MOS	TATGGAGTTTGGTGGTAA
	(SEQ ID NO: 26)	(SEQ ID NO: 572)
88:276A	CDK4	GGAAGGGTCGTTTAAGGG
	(SEQ ID NO: 5)	(SEQ ID NO: 563)
88:276B	CDK4	GGAAGGGTTGTTTAAGGG
	(SEQ ID NO: 5)	(SEQ ID NO: 564)
89:530A	MOS	TTTATTGTCGTATTGGAG
	(SEQ ID NO: 26)	(SEQ ID NO: 595)
89:530B	MOS	TTTATTGTTGTATTGGAG
	(SEQ ID NO: 26)	(SEQ ID NO: 596)

Table 6: Differentiation of MCL from Follicular Lymphoma(male samples only)

No:	Gene	Oligo:
2157:1808A	MLH1	GTAGTAGTCGTTTTAGGG
	(SEQ ID NO: 25)	(SEQ ID NO: 559)

No:	Gene	Oligo:
2428:2120A18	ESR	TGGAGGTTCGGGAGTTTA
8	(SEQ ID NO: 34)	(SEQ ID NO: 525)
2428:2120B18		TGGAGGTTTGGGAGTTTA
	(SEQ ID NO: 34)	(SEQ ID NO: 526)
1010:753 A188		AGTTTAGTCGGTTGGTAT
	(SEQ ID NO: 11)	(SEQ ID NO: 367)
1010:753 B188	MYC1	AGTTTAGTTGGTTGTAT
	(SEQ ID NO: 11)	(SEQ ID NO: 368)
130:269 A188		GGTTAGGTCGTAGTATTG
	(SEQ ID NO: 8)	(SEQ ID NO: 345)
130:269 B188	GPIb beta	GGTTAGGTTGTAGTATTG
	(SEQ ID NO: 8)	(SEQ ID NO: 346)
2322:996 A188		GAGTGTTCGCGTTTTGGG
	(SEQ ID NO: 31)	(SEQ ID NO: 593)
2322:996 B188		GAGTGTTTGTGTTTTGGG
	(SEQ ID NO: 31)	(SEQ ID NO: 594)
2157:1808B	MLH1	GTAGTAGTTTTTAGGG
	(SEQ ID NO: 25)	(SEQ ID NO: 560)
2013:1990A	BCL2	AGTTGGGGCGAGAGGTGT
	(SEQ ID NO: 15)	(SEQ ID NO: 567)
2013:1990B	BCL2	AGTTGGGGTGAGAGGTGT
	(SEQ ID NO: 15)	(SEQ ID NO: 568)
2033:1847A	CDKN1A	AGTTGGTTCGGCGTTGGG
	(SEQ ID NO: 17)	(SEQ ID NO: 589)
2033:1847B	CDKN1A	AGTTGGTTTGGG
,	(SEQ ID NO: 17)	(SEQ ID NO: 590)
2494:1989A	BAK1	GGAGTTTCGCGGGTTTTT
	(SEQ ID NO: 36)	(SEQ ID NO: 565)
2494:1989B	BAK1	GGAGTTTTGTGGGTTTTT
,	(SEQ ID NO: 36)	(SEQ ID NO: 566)
2036:2234A	CDKN2B	AGAGAGTGCGTCGGAGTA
	(SEQ ID NO: 20)	(SEQ ID NO: 575)
2036:2234B	CDKN2B	AGAGAGTGTTGGAGTA
	(SEQ ID NO: 20)	(SEQ ID NO: 576)
130:175A	GPIb beta	GTGGGAGCGGAAGTTTGA
	(SEQ ID NO: 8)	(SEQ ID NO: 561)
130:175B	GPIb beta	GTGGGAGTGGAAGTTTGA
	(SEQ ID NO: 8)	(SEQ ID NO: 562)
2322:1213A	TP73	GAGTGTTCGCGTTTTGGG
	(SEQ ID NO: 31)	(SEQ ID NO: 593)
2322:1213B	TP73	GAGTGTTTGTGTTTTGGG
-0-12-13-13	(SEQ ID NO: 31)	(SEQ ID NO: 594)
37:168A	AR	AGAGGTTGCGTTTTAGAG
- :	(SEQ ID NO: 4)	(SEQ ID NO: 569)
37:168B	AR	AGAGGTTGTGTTTTAGAG
	(SEQ ID NO: 4)	(SEQ ID NO: 570)
30:165A	GPIb beta	TTTGAGAGCGGGTGGGAG
0,10011	(SEQ ID NO: 8)	(SEQ ID NO: 579)
30:165B	GPIb beta	TTTGAGAGTGGGTGGGAG
	CARO OCIA	DAUDU LUUU LUutuu Lutuu

No:	Gene	Oligo:
2157:1797A	MLH1	TTAGGTAGCGGGTAGTAG
	(SEQ ID NO: 25)	(SEQ ID NO: 573)
2157:1797B	MLH1	TTAGGTAGTGGTAGTAG
	(SEQ ID NO: 25)	(SEQ ID NO: 574)
89:530A	MOS	TTTATTGTCGTATTGGAG
	(SEQ ID NO: 26)	(SEQ ID NO: 595)
89:530B	MOS	TTTATTGTTGTATTGGAG
	(SEQ ID NO: 26)	(SEQ ID NO: 596)
2322:1082A	TP73	TTTGGTGCGCGTAGAGAA
	(SEQ ID NO: 31)	(SEQ ID NO: 581)
2322:1082B	TP73	TTTGGTGTGTAGAGAA
	(SEQ ID NO: 31)	(SEQ ID NO: 582)
2036:2183A	CDKN2B	TAGAAGGACGACGGGAGG
	(SEQ ID NO: 20)	(SEQ ID NO: 577)
2036:2183B	CDKN2B	TAGAAGGATGATGGGAGG
	(SEQ ID NO: 20)	(SEQ ID NO: 578)

Table 7: Differentiation of FL from CLL/SLL

No:	Gene	Oligo:
2428:2120A18	ESR	TGGAGGTTCGGGAGTTTA
	(SEQ ID NO: 34)	(SEQ ID NO: 525)
2428:2120B188	ESR	TGGAGGTTTGGGAGTTTA
	(SEQ ID NO: 34)	(SEQ ID NO: 526)
1012:2491A18	ELK1	GAAGGGTTCGTTTTTAA
	(SEQ ID NO: 12)	(SEQ ID NO: 375)
1012:2491B188	ELK1	GAAGGGTTTGTTTTTAA
	(SEQ ID NO: 12)	(SEQ ID NO: 376)
2033:1847A	CDKN1A	AGTTGGTTCGGCGTTGGG
	(SEQ ID NO: 17)	(SEQ ID NO: 589)
2033:1847B	CDKN1A	AGTTGGTTTGGG
	(SEQ ID NO: 17)	(SEQ ID NO: 590)
130:165A	GPIb beta	TTTGAGAGCGGGTGGGAG
	(SEQ ID NO: 8)	(SEQ ID NO: 579)
130:165B	GPIb beta	TTTGAGAGTGGGTGGGAG
	(SEQ ID NO: 8)	(SEQ ID NO: 580)
168:1477A	MYOD1	GTGTTAGTCGTTTAGGGT
	(SEQ ID NO: 9)	(SEQ ID NO: 597)
168:1477B	MYOD1	GTGTTAGTTTTAGGGT
	(SEQ ID NO: 9)	(SEQ ID NO: 598)
130:386A	GPIb beta	ATGGGTTTCGGTGAGTTT
	(SEQ ID NO: 8)	(SEQ ID NO: 599)
130:386B	GPIb beta	ATGGGTTTTGGTGAGTTT
	(SEQ ID NO: 8)	(SEQ ID NO: 600)
2036:2183A	CDKN2B	TAGAAGGACGACGGAGG
	(SEQ ID NO: 20)	(SEQ ID NO: 577)
2036:2183B	CDKN2B	TAGAAGGATGATGGGAGG
	(SEQ ID NO: 20)	(SEQ ID NO: 578)

2013:1990A		Oligo:
Z015:1990A	BCL2	AGTTGGGGCGAGAGGTGT
	(SEQ ID NO: 15)	(SEQ ID NO: 567)
2013:1990B	BCL2	AGTTGGGGTGAGAGGTGT
	(SEQ ID NO: 15)	(SEQ ID NO: 568)
	TP73	AAGTTACGGGTTTTATTG
	(SEQ ID NO: 31)	(SEQ ID NO: 587)
	TP73	AAGTTATGGGTTTTATTG
. ((SEQ ID NO: 31)	(SEQ ID NO: 588)
96:508A	RB1	GTGTATTTCGGTTTGGAG
	SEQ ID NO: 6)	(SEQ ID NO: 601)
	RB1	GTGTATTTGGTTTGGAG
	SEQ ID NO: 6)	(SEQ ID NO: 602)
2157:1797A	MLH1	TTAGGTAGCGGGTAGTAG
- (SEQ ID NO: 25)	(SEQ ID NO: 573)
	MLH1	TTAGGTAGTGGTAGTAG
	SEQ ID NO: 25)	(SEQ ID NO: 574)
88:276A	CDK4	GGAAGGGTCGTTTAAGGG
	SEQ ID NO: 5)	(SEQ ID NO: 563)
88:276B	CDK4	GGAAGGGTTGTTTAAGGG
	SEQ ID NO: 5)	(SEQ ID NO: 564)
130:175A	GPIb beta	GTGGGAGCGGAAGTTTGA
	SEQ ID NO: 8)	(SEQ ID NO: 561)
130:175B	GPIb beta	GTGGGAGTGGAAGTTTGA
	SEQ ID NO: 8)	(SEQ ID NO: 562)
2494:1989A]	BAK1	GGAGTTTCGCGGGTTTTT
	SEQ ID NO: 36)	(SEQ ID NO: 565)
2494:1989B I	BAK1	GGAGTTTTGTGGGTTTTT
(5	SEQ ID NO: 36)	(SEQ ID NO: 566)
2036:2234A	CDKN2B	AGAGAGTGCGTCGGAGTA
	SEQ ID NO: 20)	(SEQ ID NO: 575)
2036:2234B	CDKN2B	AGAGAGTGTTTGGAGTA
	SEQ ID NO: 20)	(SEQ ID NO: 576)
87:168A	AR	AGAGGTTGCGTTTTAGAG
	SEQ ID NO: 4)	(SEQ ID NO: 569)
87:168B	AR	AGAGGTTGTGTTTTAGAG
(5	SEQ ID NO: 4)	(SEQ ID NO: 570)
89:451A N	MOS	TATGGAGTTCGGTGGTAA
	SEQ ID NO: 26)	(SEQ ID NO: 571)
89:451B	MOS	TATGGAGTTTGGTGGTAA
	SEQ ID NO: 26)	(SEQ ID NO: 572)
89:530A N	MOS	TTTATTGTCGTATTGGAG
(SEQ ID NO: 26)	(SEQ ID NO: 595)
,	MOS	TTTATTGTTGTATTGGAG
(S	SEQ ID NO: 26)	(SEQ ID NO: 596)

Table 8: Differentiation of FL from CLL/SLL(male samples only)

No:	Gene	Oligo;
	134.74	0.79.

No:	Gene	Oligo:	
2494:1989A	BAK1	GGAGTTTCGCGGGTTTTT	
	(SEQ ID NO: 36)	(SEQ ID NO: 565)	
2494:1989B	BAK1	GGAGTTTTGTGGGTTTTT	
	(SEQ ID NO: 36)	(SEQ ID NO: 566)	
130:175A	GPIb beta	GTGGGAGCGGAAGTTTGA	
	(SEQ ID NO: 8)	(SEQ ID NO: 561)	
130:175B	GPIb beta	GTGGGAGTGGAAGTTTGA	
	(SEQ ID NO: 8)	(SEQ ID NO: 562)	
87:168A	AR	AGAGGTTGCGTTTTAGAG	
	(SEQ ID NO: 4)	(SEQ ID NO: 569)	
2428:2120A18	ESR .	TGGAGGTTCGGGAGTTTA	
8	(SEQ ID NO: 34)	(SEQ ID NO: 525)	
2428:2120B188	ESR	TGGAGGTTTGGGAGTTTA	
	(SEQ ID NO: 34)	(SEQ ID NO: 526)	
87:168B	AR	AGAGGTTGTGTTTTAGAG	
	(SEQ ID NO: 4)	(SEQ ID NO: 570)	
2036:2234A	CDKN2B	AGAGAGTGCGTCGGAGTA	
	(SEQ ID NO: 20)	(SEQ ID NO: 575)	
2036:2234B	CDKN2B	AGAGAGTGTGTTGGAGTA	
	(SEQ ID NO: 20)	(SEQ ID NO: 576)	

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Patent Claims

- 1. A method for detecting and differentiating between lymphoid cell proliferative disorders associated with at least one gene and/or their regulatory regions from the group comprising MDR1, CSNK2B, EGR4, AR, CDK4, RB1, CDC25A, GPIb beta, MYOD1, CDH3, MYCL1, ELK1, ABL1, APC, BCL2, CDH1, CDKN1A, CDKN1B, CDKN2a, CDKN2B, FOS, GSTP1, HIC-1, MGMT, MLH1, MOS, MYC, PTEN, RBL2, TGFBR2, TP73, CDKN1C, GSK3ß, ESR1, APAF1, BAK1, BAX and HOXA5 in a subject, said method comprising contacting a target nucleic acid in a biological sample obtained from said subject with at least one reagent or a series of reagents, wherein said reagent or series of reagents, distinguishes between methylated and non methylated CpG dinucleotides within the target nucleic acid.
- 2. The method according to claim 1 wherein, said method differentiates between at least two members of the following group of medical conditions: diffuse large B-cell lymphoma, mantle cell lymphoma, chronic lymphocytic leukaemia, small lymphocytic lymphoma and follicular lymphoma.
- 3. The method according to claim 1 wherein, said method differentiates between mantle cell lymphoma and chronic lymphocytic leukemia or small lymphocytic lymphoma.
- 4. The method according to claim 1 wherein, said method differentiates between mantle cell lymphoma and follicular lymphoma.
- 5. The method according to Claims 2, 3 and 4 wherein, said method differentiates between lymphomas originating in subjects of the same sex.
- 6. The method according to any one of Claims 1 to 5 comprising the following steps:
 - obtaining a biological sample containing genomic DNA,
 - extracting the genomic DNA,
 - converting cytosine bases in the genomic DNA sample which are unmethylated at the 5-position, by treatment, to uracil or another base which is dissimilar to cytosine in terms of base pairing behaviour,

- fragments of the pretreated genomic DNA are amplified, and
- identification of the methylation status of one or more cytosine positions.
- 7. The method according to claim 6, characterised in that the reagent is a solution of bisulfite, hydrogen sulfite or disulfite.
- 8. The method as recited in Claims 6 or 7, characterised in that the amplification is carried out by means of the polymerase chain reaction (PCR).
- 9. The method as recited in one of the Claims 6 through 8, characterised in that the amplification is carried out by means of a heat-resistant DNA polymerase.
- 10. The method as recited in one of the Claims 6 through 9, characterised in that more than ten different fragments having a length of 100 2000 base pairs are amplified.
- 11. The method as recited in one of claims 6 through 10, wherein the amplification step is carried out using a set of primer oligonucleotides comprising SEQ ID NO: 213 through SEQ ID NO: 290.
- 12. The method as recited in one of the Claims 6 through 11, characterised in that the amplification of several DNA segments is carried out in one reaction vessel.
- 13. The method as recited in one of Claims 6 through 12, characterised in that the amplification step preferentially amplifies DNA which is of particular interest in healthy and/or diseased lymphoid tissues, based on the specific genomic methylation status of lymphoid tissue, as opposed to background DNA.
- 14. The method according to one of Claims 6 through 13, characterised in that the methylation status within at least one gene and/or their regulatory regions from the group comprising MDR1, CSNK2B, EGR4, AR, CDK4, RB1, CDC25A, GPIb beta, MYOD1, CDH3, MYCL1, ELK1, ABL1, APC, BCL2, CDH1, CDKN1A, CDKN1B, CDKN2a, CDKN2B, FOS, GSTP1, HIC-1, MGMT, MLH1, MOS, MYC, PTEN, RBL2, TGFBR2, TP73, CDKN1C, GSK3ß, ESR1, APAF1, BAK1, BAX and HOXA5 is detected by hybridisation of each amplificate to an oligonucleotide or peptide nucleic acid (PNA)-oligomer.

- 15. The method according to claim 14, characterised in that the olignonucleotide or peptide nucleic acid (PNA)-oligomer is taken from the group comprising SEQ ID NO: 291 to SEQ ID NO: 602.
- 16. The method according to Claims 6 through 15, characterised in that the amplificates are labelled.
- 17. The method as recited in Claim 16, characterised in that the labels of the amplificates are selected from the group consisting of radionuclides, fluorescence labels, and/or detachable molecule fragments having a typical mass which are detected in a mass spectrometer.
- 18. The method as recited in one of the Claims 6 through 17, characterised in that the amplificates or fragments of the amplificates are detected in the mass spectrometer.
- 19. The method as recited in one of the Claims 17 and 18, characterised in that the produced fragments have a single positive or negative net charge.
- 20. The method as recited in one of the Claims 17 through 19, characterised in that detection is carried out and visualised by means of matrix assisted laser desorption/ionisation mass spectrometry (MALDI) or using electron spray mass spectrometry (ESI).
- 21. The method according to Claims 1 through 5 comprising the following steps;
 - a) obtaining a biological sample containing genomic DNA,
 - b) extracting said genomic DNA from said sample,
 - c) digesting the genomic DNA which comprises at least one or more CpGs of the genes MDR1, CSNK2B, EGR4, AR, CDK4, RB1, CDC25A, GPIb beta, MYOD1, CDH3, MYCL1, ELK1, ABL1, APC, BCL2, CDH1, CDKN1A, CDKN1B, CDKN2a, CDKN2B, FOS, GSTP1, HIC-1, MGMT, MLH1, MOS, MYC, PTEN, RBL2, TGFBR2, TP73, CDKN1C, GSK3ß, ESR1, APAF1, BAK1, BAX and HOXA5 with one or more methylation sensitive restriction enzymes, and
 - d) detecting of the DNA fragments generated in the digest of step c).
- 22. The method according to Claim 21, wherein the DNA digest is amplified prior to Step d).

- 23. The method as recited in Claim 22, characterised in that the amplification is carried out by means of the polymerase chain reaction (PCR).
- 24. The method as recited in one of the Claims 22 and/or 23, characterised in that the amplification of more than one DNA fragment is carried out in one reaction vessel.
- 25. The method as recited in one of the Claims 22 through 24, characterised in that the polymerase is a heat-resistant DNA polymerase.
- 26.An isolated nucleic acid of a pretreated genomic DNA according to one of the sequences taken from the group comprising SEQ ID NO: 61 to SEQ ID NO: 212 and sequences complementary thereto.
- 27.An oligomer, in particular an oligonucleotide or peptide nucleic acid (PNA)-oligomer, said oligomer comprising at least one base sequence of at least 10 nucleotides which hybridises to or is identical to a pretreated genomic DNA according to one of the SEQ ID NO: 61 to SEQ ID NO: 212 according to Claim 26.
- 28. The oligonucleotide as recited in Claim 27; wherein the base sequence includes at least one CpG or TpG dinucleotide sequence.
- 29. The oligonucleotide as recited in Claim 28; characterized in that the cytosine of the at least one CpG or TpG dinucleotide is/are located approximately in the middle third of the oligomer.
- 30.An oligomer, in particular an oligonucleotide or peptide nucleic acid (PNA)-oligomer, according to one of the sequences taken from the group comprising SEQ ID NO: 291 to SEQ ID NO: 602.
- 31.A set of oligonucleotides, comprising at least two oligonucleotides according to any of Claims 27 through 30.

- 32.A set of oligonucleotides, comprising at least two oligonucleotides according to SEQ ID NO: 559 to SEQ ID NO: 578, and 375 and 525.
- 33.One or more isolated nucleic acid(s) taken from the group according to SEQ ID NO: 39 to SEQ ID NO: 48.
- 34.A set of oligonucleotides, comprising at least two oligonucleotides according to SEQ ID NO: 561, 562, 565, 566, 569, 570, 573 to 580, 525, 367, 375, 559, 345, and 593.
- 35.One or more isolated nucleic acid(s) taken from the group according to SEQ ID NO: 40, 42, 44, and 46 to 49.
- 36.A set of oligonucleotides, comprising at least two oligonucleotides according to SEQ ID NO: 561 to 580, 587 to 590, 595 to 602, 525, and 375.
- 37.One or more isolated nucleic acid(s) taken from the group according to SEQ ID NO: 40 to 49, 53, 54, and 57 to 60.
- 38.A set of oligonucleotides, comprising at least two oligonucleotides according to SEQ ID NO: 561, 562, 565, 566, 569, 570, 575, 576, and 525.
- 39. One or more isolated nucleic acid(s) taken from the group according to SEQ ID NO: 40, 42, 44 and 47.
- 40.A set of oligonucleotides, comprising at least two oligonucleotides according to SEQ ID NO: 559 to 596, 525, 367, 375, 501, 537, and 601.
- 41.One or more isolated nucleic acid(s) taken from the group according to SEQ ID NO: 39 to 57.
- 42.A set of oligonucleotides, comprising at least two oligonucleotides according to SEQ ID NO: 559 to 562, 565 to 570, 573 to 582, 589, 590, 593 to 596, 525, 367, 345, and 503.

- 43. One or more isolated nucleic acid(s) taken from the group according to SEQ ID NO: 39, 40, 42 to 44, 46 to 50, 54, 56 and 57.
- 44. A set of oligomers, peptide nucleic acid (PNA)-oligomers and/or isolated mucleic acids as recited in Claims 31 through 43, comprising oligomers for detecting the methylation state of all CpG dinucleotides within one or more of the sequences according to SEQ ID NO: 1 to SEQ ID NO: 38 and sequences complementary thereto.
- 45.Use of a set of oligomers or peptide nucleic acid (PNA)-oligomers according to any of claims 27 through 32, 34, 36, 38, 40, and 42 as probes for determining the cytosine methylation state and/or single nucleotide polymorphisms (SNPs) of sequences according to SEQ ID NO: 1 to SEQ ID NO: 38 and sequences complementary thereto.
- 46.Use of a set of oligonucleotides according to Claim 32 or nucleic acid(s) according to Claim 33 for the differentiation of at least two members of the following group of medical conditions: diffuse large B-cell lymphoma, mantle cell lymphoma, chronic lymphocytic leukaemia, small lymphocytic lymphoma and follicular lymphoma.
- 47.Use of a set of oligonucleotides according to Claim 34 or nucleic acid(s) according to Claim 35 for the differentiation of at least two members of the following group of medical conditions: diffuse large B-cell lymphoma, mantle cell lymphoma, chronic lymphocytic leukaemia, small lymphocytic lymphoma and follicular lymphoma wherein said lymphomas are of male origin.
- 48.Use of a set of oligonucleotides according to Claim 36 or nucleic acid(s) according to Claim 37 for the differentiation between follicular lymphoma and chronic lymphocytic leukaemia or small lymphocytic lymphoma.
- 49.Use of a set of oligonucleotides according to Claim 38 or nucleic acid(s) according to Claim 39 for the differentiation between follicular lymphoma and chronic lymphocytic leukaemia or small lymphocytic lymphoma wherein said lymphomas are of male origin.
- 50.Use of a set of oligonucleotides according to Claim 40 or nucleic acid(s) according to Claim 41 for the differentiation between mantle cell lymphoma and follicular lymphoma.

- 51.Use of a set of oligonucleotides according to Claim 42 or nucleic acid(s) according to Claim 43 for the differentiation between mantle cell lymphoma and follicular lymphoma wherein said lymphomas are of male origin.
- 52.Use of a set of at least two oligonucleotides or peptide nucleic acid (PNA)-oligomers as recited in Claim 27 as primer oligonucleotides for the amplification of DNA sequences of one of SEQ ID NO: 61 to SEQ ID NO: 212 according to Claim 26 and/or sequences complementary thereto and segments thereof.
- 53. Use of a pretreated genomic DNA according to claim 26 for the determination of the methylation status of a corresponding genomic DNA and/or detection of single nucleotide polymorphisms (SNPs).
- 54. A set of oligonucleotides or peptide nucleic acid (PNA)-oligomers as recited in Claims 31, 32, 34, 36, 38, 40 or 42, characterised in that at least one oligonucleotide is bound to a solid phase.
- 55. A set of oligonucleotides or peptide nucleic acid (PNA)-oligomers as recited in Claims 31, 32, 34, 36, 38, 40 or 42, characterised in that all members of the set are bound to a solid phase.
- 56.A method for manufacturing an arrangement of different oligomers or peptide nucleic acid (PNA)-oligomers (array) for analysing diseases associated with the corresponding genomic methylation status of the CpG dinucleotides within one of the SEQ ID NO: 1 to SEQ ID NO: 38 and sequences complementary thereto, wherein at least one oligomer according to any of the Claims 31, 32, 34, 36, 38, 40 or 42 is coupled to a solid phase.
- 57.An arrangement of different oligomers or peptide nucleic acid (PNA)-oligomers (array) obtained according to claim 56.
- 58.An array of different oligonucleotide- and/or PNA-oligomer sequences as recited in Claim 57, characterised in that these are arranged on a plane solid phase in the form of a rectangular or hexagonal lattice.

- 59. A nucleic acid or peptide nucleic acid array for the analysis of lymphoid cell proliferative disorders associated with the methylation state of genes comprising at least one nucleic acid according to one of the preceding claims.
- 60. The array as recited in any of the Claims 57 through 59, characterised in that the solid phase surface is composed of silicon, glass, polystyrene, aluminium, steel, iron, copper, nickel, silver, or gold.
- 61.A kit comprising a bisulfite (= disulfite, hydrogen sulfite) reagent as well as oligonucleotides and/or PNA-oligomers according to one of the Claims 27 through 43.
- 62. The use of oligonucleotides or peptide nucleic acid (PNA)-oligomers according to SEQ ID NO: 61 through SEQ ID NO: 602 for the detection of a predisposition to, differentiation between subclasses, diagnosis, prognosis, treatment and/or monitoring of lymphoid cell proliferative disorders.
- 63.Use of a DNA sequence according to one of the sequences taken from the group comprising SEQ ID NO: 61 to SEQ ID NO: 212 and sequences complementary thereto for the analysis of cytosine methylation within said nucleic acid for the detection of a predisposition to, differentiation between subclasses, diagnosis, prognosis, treatment and/or monitoring of lymphoid cell proliferative disorders.

Figure 1

1	2 .	3
UM-L1	MCL	M
· UM-L2	MCL	. M
UM-L3	MCL	M
UM-L4	MCL	M
UM-L5	MCL	M
UM-L6	MCL	M
UM-L7	MCL	M
UM-L8	MCL	M
UM-L9	MCL	M
UM-L10	MCL	M
UM-L11	CLL/SLL	M
UM-L12	CLL/SLL	<u>M</u> ¬
UM-L13	CLL/SLL	M
UM-L14	CLL/SLL	F
UM-L15 UM-L16	CLL/SLL CLL/SLL	M
UM-L16 UM-L17	FL I	F
UM-L18	FL I	M F
UM-L19	FL I	F
UM-L20	FL I	<u>г</u> М
UM-L21	FL I	M
UM-L22	FL I	M
UM-L23	FL I	M
UM-L24	CLL/SLL	F
UM-L25	CLL/SLL	M
UM-L26	CLL/SLL	M
UM-L27	CLL/SLL	F
UM-L28	CLL/SLL	M
UM-L29	MCL ·	M
UM-L30	MCL	M
UM-L32	MCL	M
UM-L33	MCL	M
UM-L34	CLL/SLL	M
UM-L35	FL I	M
UM-L36	MCL	M
UM-L37	CLL/SLL	M
UM-L38	CLL/SLL	M
UM-L39	CLL/SLL	M
UM-L45	FL I	F
UM-L52	FL I	F
UM-L53	FL I	M
UM-L54 UM-L55	FL I	M
UM-L56	FL I	<u>M</u>
UM-L58	FL I FL I	M
UM-L59	· · · · · · · · · · · · · · · · · · ·	F
O1v1-L39	FL I	F

1	2	3
UM-L60	FL I	M
	FL I	F
UM-L61		
UM-L62	FL I	F
UM-L63	FLI	M
UM-L64	FL I	M
UM-L65	FL I	M
UM-L66	FL I	F
UM-L67	FL I	. M
UM-L68	FL I	M
UM-L69	FL I	· F
UM-L70	DLBCL	F
UM-L71	DLBCL	M
UM-L72	DLBCL	M
UM-L73	DLBCL	F
UM-L74	DLBCL	M
UM-L75	DLBCL	M
UM-L76	DLBCL	M
UM-L77	DLBCL	M
UM-L78	DLBCL	F
UM-L79	DLBCL	M
UM-L80	DLBCL	F
UM-L81	DLBCL	. F
UM-L82	DLBCL	M
UM-L83	DLBCL	M

FIGURE 2

MCL&DLBČL&ČLL/ŠLL

FIGURE 3

ACL&DIBCL&CLL/SIL

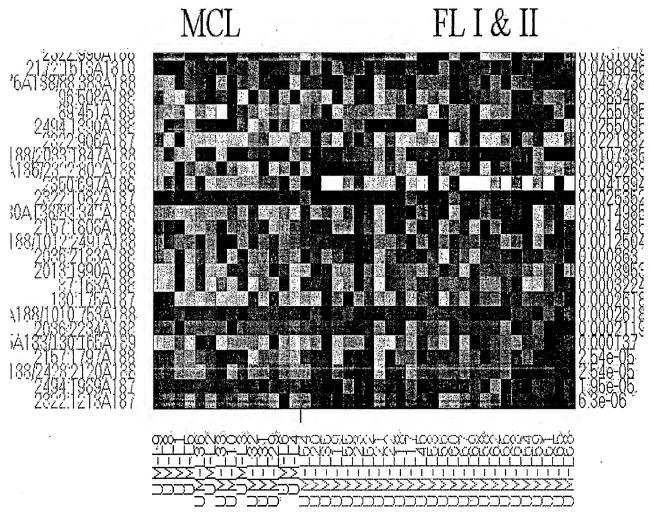


Figure 4

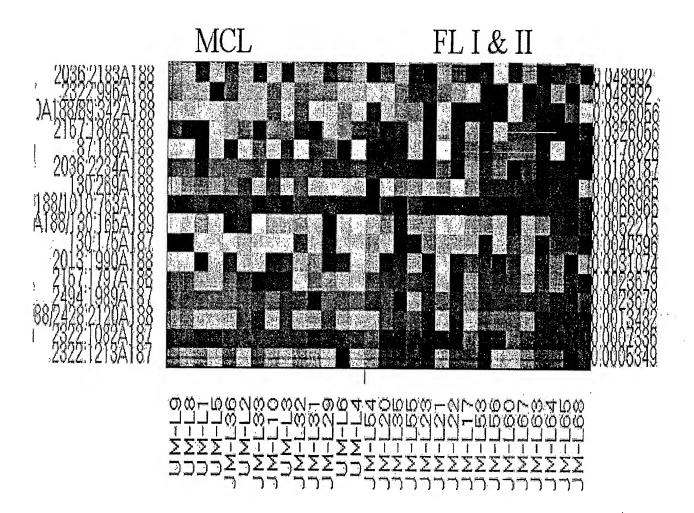


Figure 5

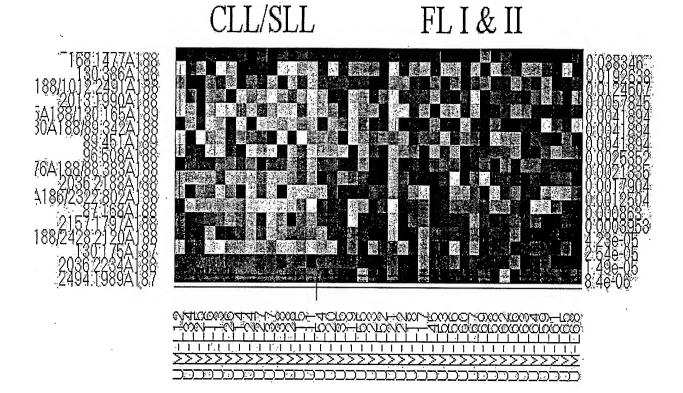
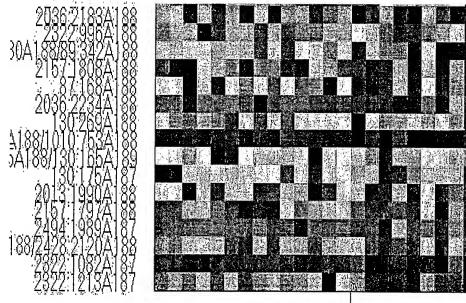


Figure 6

CLL/SLL

FLI&II



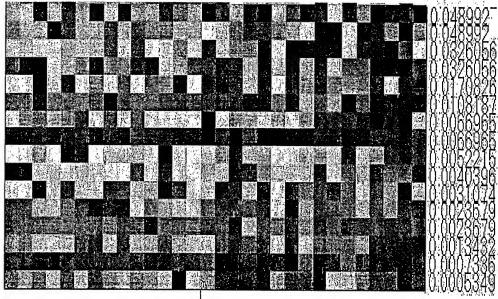


Figure 7

Sequence listing

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<211> 2932

<212> DNA

<213> Homo Sapiens

<400> 1

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